361

VOL. III APRIL, 1908 No. 2

THE PHILIPPINE
JOURNAL OF SCIENCE

EDITED BY

PAUL C. FREER, M. D., PH. D.

CO-EDITOR

RICHARD P. STRONG, PH. B., M. D.

WITH THE COLLABORATION OF

VICTOR G. HEISER, M. D.; W. E. MUSGRAVE, M. D.
HARRY T. MARSHALL, A. B., M. D.
JOHN R. McDILL, M. D.; FERNANDO CALDERON, M. D.
JOSE ALBERT, M. D.; PHILIP K. GILMAN, A. B., M. D.
PHILIP E. GARRISON, B. A., M. D.

PUBLISHED BY

THE BUREAU OF SCIENCE

OF THE

GOVERNMENT OF THE PHILIPPINE ISLANDS

B. MEDICAL SCIENCES

MANILA
BUREAU OF PRINTING
1908

PREVIOUS PUBLICATIONS OF THE BUREAU OF GOVERNMENT LABORATORIES.

No. 1, 1902, Biological Laboratory.—Preliminary Report of the Appearance in the Philippine Islands of a Disease Clinically Resembling Glanders. By R. P. Strong, M. D.

No. 2, 1902, Chemical Laboratory.—The Preparation of Benzoyl-Acetyl Peroxide and Its Use as an Intestinal Antiseptic in Cholera and Dysentery. Preliminary Notes. By Paul C. Freer, M. D., Ph. D.

No. 3, 1903, Biological Laboratory.—A Preliminary Report on Trypanosomiasis of Horses in the Philippine Islands. By W. E. Musgrave, M. D., and Norman E. Williamson.

No. 4, 1903, Serum Laboratory.—Preliminary Report on the Study of Rinderpest of Cattle and Carabaos in the Philippine Islands. By James W. Jobling, M. D.

No. 5, 1903, Biological Laboratory.—Trypanosoma and Trypanosomiasis, with Special Reference to Surra in the Philippine Islands. By W. E. Musgrave, M. D., and Moses T. Clegg.

No. 6, 1903.—New or Noteworthy Plants, I. The American Element in the Philippine Flora. By Elmer D. Merrill, Botanist. (Issued January 20, 1904.)

No. 7, 1903, Chemical Laboratory.—The Gutta Percha and Rubber of the Philippine Islands. By Penoyer L. Sherman, jr., Ph. D.

No. 8, 1903.—A Dictionary of the Plant Names of the Philippine Islands. By Elmer D. Merrill, Botanist.

No. 9, 1903, Biological and Serum Laboratories.—A Report on Hæmorrhagic Septicæmia in Animals in the Philippine Islands. By Paul G. Woolley, M, D., and J. W. Jobling, M. D.

No. 10, 1903, Biological Laboratory.—Two Cases of a Peculiar Form of Hand Infection (Due to an Organism Resembling the Koch-Weeks Bacillus). By John R. McDill, M. D., and Wm. B. Wherry, M. D.

No. 11, 1903, Biological Laboratory.—Entomological Division, Bulletin No. 1: Preliminary Bulletin on Insects of the Cacao. (Prepared Especially for the Benefit of Farmers.) By Charles S. Banks, Entomologist.

No. 12, 1903, Biological Laboratory.—Report on Some Pulmonary Lesions Produced by the Bacillus of Hæmorrhagic Septicæmia of Carabaos. By Paul G. Woolley, M. D.

No. 13, 1904, Biological Laboratory.—A Fatal Infection by a Hitherto Undescribed Chromogenic Bacterium: Bacillus Aureus Fœtidus. By Maximilian Herzog, M. D.

No. 14, 1904.—Serum Laboratory: Texas Fever in the Philippine Islands and the Far East. By J. W. Jobling, M. D., and Paul G. Woolley, M. D. *Biological Laboratory:* Entomological Division, Bulletin No. 2: The Australian Tick (Boophilus Australis Fuller) in the Philippine Islands. By Charles S. Banks, Entomologist.

No. 15, 1904, Biological and Serum Laboratories.—Report on Bacillus Violaceus Manilæ: A Pathogenic Micro-Organism. By Paul G. Woolley, M. D.

No. 16, 1904, Biological Laboratory.—Protective Inoculation Against Asiatic Cholera: An Experimental Study. By Richard P. Strong, M. D.

No. 17, 1904.—New or Noteworthy Philippine Plants, II. By Elmer D. Merrill, Botanist.

No. 18, 1904, Biological Laboratory.—I. Amebas: Their Cultivation and Etiologic Significance. By W. E. Musgrave, M. D., and Moses T. Clegg. II. The Treatment of Intestinal Amœbiasis (Amœbic Dysentery) in the Tropics. By W. E. Musgrave, M. D.

No. 19, 1904, Biological Laboratory.—Some Observations on the Biology of the Cholera Spirillum. By W. B. Wherry, M. D.

No. 20, 1904.—Biological Laboratory: I. Does Latent or Dormant Plague Exist Where the Disease is Endemic? By Maximilian Herzog, M. D., and Charles B. Hare. *Serum Laboratory:* II. Broncho-Pneumonia of Cattle: Its Association with B. Bovisepticus. By Paul G. Woolley, M. D., and Walter Sorrell, D. V. S. III. Pinto (Paño Blanco). By Paul G. Woolley, M. D. *Chemical Laboratory:* IV. Notes on Analysis of the Water from the Manila Water Supply. By Charles L. Bliss, M. S. *Serum Laboratory:* V. Frambœsia: Its Occurrence in Natives in the Philippine Islands. By Paul G. Woolley, M. D.

No. 21, 1904, Biological Laboratory.—Some Questions Relating to the Virulence of Micro-Organisms with Particular Reference to Their Immunizing Powers. By Richard P. Strong, M. D.

No. 22, 1904, Bureau of Government Laboratories.—I. A Description of the New Buildings of the Bureau of Government Laboratories. By Paul C. Freer, M. D., Ph. D. II. A Catalogue of the Library of the Bureau of Government Laboratories. By Mary Polk, Librarian.

No. 23, 1904, Biological Laboratory.—Plague: Bacteriology, Morbid Anatomy, and Histopathology (Including a Consideration of Insects as Plague Carriers). By Maximilian Herzog, M. D.

No. 24, 1904, Biological Laboratory.—Glanders: Its Diagnosis and Prevention (Together with a Report on Two Cases of Human Glanders Occurring in Manila and Some Notes on the Bacteriology and Polymorphism of Bacterium Mallei). By William B. Wherry, M. D.

No. 25, 1904.[1]—Birds from the Islands of Romblon, Sibuyan, and Cresta de Gallo. By Richard C. McGregor.

No. 26, 1904, Biological Laboratory.—The Clinical and Pathological Significance of Balantidium Coli. By Richard P. Strong, M. D.

No. 27, 1904.—A Review of the Identification of the Species Described in Blanco's Flora de Filipinas. By Elmer D. Merrill, Botanist.

No. 28, 1904.—I. The Polypodiaceæ of the Philippine Islands. II. Edible Philippine Fungi. By Edwin B. Copeland, Ph. D.

No. 29, 1904.—I. New or Noteworthy Philippine Plants, III. II. The Source of Manila Elemi. By Elmer D. Merrill, Botanist.

No. 30, 1905, Chemical Laboratory.—I. Autocatalytic Decomposition of Silver Oxide. II. Hydration in Solution. By Gilbert N. Lewis, Ph. D.

No. 31, 1905, Biological Laboratory.—I. Notes on a Case of Hæmatochyluria (Together with Some Observations on the Morphology of the Embryo Nematode, Filaria Nocturna). By William B. Wherry, M. D., and John R. McDill, M. D., Manila, P. I. II. A Search Into the Nitrate and Nitrite Content of Witte's "Peptone," with Special Reference to Its Influence on the Demonstration of the Indol and Cholera-Red Reactions. By William B. Wherry, M. D.

(Concluded on third page of cover.)

THE PHILIPPINE
JOURNAL OF SCIENCE
B. MEDICAL SCIENCES

VOL. III APRIL, 1908 No. 2

THE INFLUENCE OF SYMBIOSIS UPON THE PATHOGENICITY OF MICROÖRGANISMS[1] (THE EVOLUTION OF PARASITISM).

By W. E. MUSGRAVE.
(From the Biological Laboratory, Bureau of Science.)

CONTENTS.

INTRODUCTION.
BACTERIAL SYMBIOSIS.
ANIMAL AND BACTERIAL SYMBIOSIS.
CONCLUSIONS.

INTRODUCTION.

Symbiosis may be defined as representing all phases of association between living organisms, beginning with commensalism on the one hand and including true parasitism on the other, in which either component is influenced in nutrition, metabolism, production, or in some other manner by the presence of the other. Expressed in this broader sense, the subject should be one of interest and its study and the elucidation of its problems one of far-reaching practical importance.

There can be no reasonable doubt but that the symbiotic combinations between microörganisms are responsible for many uninterpreted phenomena in the etiology and pathology of disease, and failure properly to appreciate the existence of these combinations has been one cause of lack of progress in serum therapy. When one studies the rather meager and scattered literature on the subject, notes the promising suggestions that have been given from time to time by great masters, and carefully examines the interesting and often startling results of individual experiments, it is surprising that the subject has not been more minutely investigated. The present paper is general in character, much of it is theoretical, but in important points the conclusions are largely based upon facts.

---

[1] The President's Address, read at the Fifth Annual Meeting of the Philippine Islands Medical Association, February 26, 1908.

Nature is filled with examples of diverging biological activities. Frequently living organisms derive benefit by association; and again the presence of one organism or its products may be inimical to the life and growth of certain other forms. These facts are not confined to animals and the higher forms of plant life, but also exist so low down in the scale as to be found among the bacteria. They manifest themselves in many ways; for example, in the study of immunity the symbiosis of microörganisms has an intimate, important and much neglected place. Immunity in any instance is a very specific substance or condition and its specificity and other properties, both known and unknown, may be modified or destroyed in many ways. Among the unknown class, more light needs to be thrown upon the influence which the metabolism of both host and parasite exerts. There is evidence to show that changes in symbiosis may produce changes in metabolism and also, as a result of this, changes in the pathogenic character of parasites and in the susceptibility of hosts.

It certainly seems reasonable to assume that changes in the substances which are the stimulants calling forth the development of immunity *would result in a modification of the type of the immunity itself.* In other words, it is not inconceivable that the immunity unit is a molecule, as it were, the composition of which might be influenced by all agents reacting in its production, and that these agents might be somewhat different, even in the same disease, if they are developed under different environments. Theobald Smith [2] probably had some such idea in mind when, in his classical discussion of the parasitism of the tubercle bacillus, he stated that "In our haste to take the animal and bacterial mechanisms to pieces and to test the individual tissues and components, we have crowed out the broader view that the host fights more as a unit. We had almost forgotten to take into consideration the flexibility and adaptability of the microörganisms themselves."

It is probable that in our study of the manner in which microörganisms produce disease we have not only confined our work too closely to investigating the component parts of the invader and invaded and have not considered the two subjects as a whole, but the weakness probably extends still further and we have failed to consider the environment or symbiosis of both organisms and the influence which this environment or symbiosis may exert upon the metabolism, the toxic and the resisting properties of each, as well as the interaction of the two symbiotic elements which leads to disease or death on the one hand or to what Smith calls a "condition of balanced parasitism" on the other.

---

[2] *J. Am. Med. Ass.* (1906), 46, 1247.

For example, it is well known that certain cases of diphtheria complicated by severe streptococcus infections are not benefited by antitoxin nor are they cured by the administration of both antidiphtheritic and specific antistreptococcic serums. It is probable that one reason for failure in this instance is to be found in the fact that the toxic principle is not a simple mixture of two toxins, but that it has the nature of a new, definite, chemical compound, formed by the symbiosis of the two organisms and therefore producing a type of poison differing not only from that secreted by either, but also from any simple mixture of the two. If this should be true, it would seem altogether probable that the proper immunity unit could only be produced by the action upon animal tissues of the compound toxin radical acting as a whole.

BACTERIAL SYMBIOSIS.

Before taking up a discussion of the influences which symbiosis may exert upon the components produced by any bacterial coexistence, it is necessary to remember that there are many other elements of environment *which may exert an influence on pure species of bacteria*, similar perhaps in results to the manifestations of the same influences on such organisms in symbiosis.

Variability in the virulence of bacteria is one of their most marked features and the reason for this variability is but partially understood. We know that it may be modified by many familiar methods, but no fixed rules can be made or applied. The majority of pathogenic bacteria lose more or less of their virulence by growth upon artificial media and similarly their virulence is increased by passage through susceptible animals. However, this is not always true nor does it explain the variability. For example, there are bacteria which retain their virulence in artificial cultures and others in which this property may be considerably modified by the composition of the medium. Again, it may happen with bacteria that passage through animals will cause a permanent attenuation in some and in others increase of virulence, which for one species of animals may be coëxistent with a weakened virulence for some other species.

A satisfactory explanation of some of the alterations in virulence and of certain of the peculiarities in reactions between bacteria and animal tissues is found in what is now generally known as the hypothesis of Welch which, succinctly put by Ricketts, is as follows: "If bacterial toxins and the constituents of bacterial cells so act on the tissue cells that the latter produce bodies (antibodies) which are inimical to the bacteria, why may not the body fluids in turn so act on the bacteria that the latter produce bodies (antibodies) which are inimical to the tissue cells? Looked at from the point of view of the bacterium, as well as from that of the animal host, according to the hypothesis advanced, the struggle between the bacteria and the body cells in infections may be conceived as an immunizing contest in which each participant is stimulated by its opponent to the production of cytotoxins hostile to the other, and thereby endeavors to make itself immune against its antagonist."

If this hypothesis be true, and it certainly seems to be a reasonable one, the evolution of parasitism, even in pure cultures of bacteria would seem at least in part to depend upon the influence of other agents in supporting, or hindering, the development of either of the elements—parasite or host.

Theobald Smith[3] in discussing the parasitism of the tubercle bacillus, and bearing upon this point, "assumes as a basis for discussion a complex relationship established in time by a selective adaption between two living organisms, of which one is a parasite of the other."

"Whatever pathologic processes of a constant character are the expression of this parasitism * * * are regarded as the result of an interaction of two organisms rather than the work of one alone."

"Viewed from this standpoint, this tendency toward a state of equilibrium between host and parasite is disturbed by any change of condition which influences either parasite or host." Again, the same author believes "that the tendency of infectious disease is toward a balanced parasitism, with a reduced mortality, but not necessarily a reduced morbidity as a result."

This, Theobald Smith considers to be due to a selective action of both host and parasite. In the case of the tubercle bacillus, the very attenuated forms would become, as it were, the parasites of the sick lungs, rather than of the normal ones and would take the same position which pneumococci, streptococci and staphylococci occupy in the upper air passages.

Klein[4] in discussing the question of the evolution of bacteria from saprophytes to parasites concludes that "there is evidence to show that an ordinary saprophyte under altered conditions can produce pathogenic effect, thriving in the tissues of an animal; and that a microbe, under one condition an ordinary saprophyte, can raise itself not only to parasitic life, causing pathogenic action, but further to a parasitic action which is as specific as that of the *Bacillus anthracis* or *Bacillus influenzæ.*"

There are numerous additional facts which tend to support the evolutionary theory of parasitism. For example; under different circumstances the tubercle bacillus may manifest any rôle from the saprophyte to the most exquisite and highly specialized tissue parasite, and there is evidence tending to support the hypothesis that the higher parasitism results from an evolution as it were, produced by many factors, of which symbiosis is one. Most of the primary lesions brought about by the bacillus are formed in or near the skin or mucous surfaces and are probably influenced symbiotically or otherwise by the complex bacterial flora constantly present in these places and by the resulting modified tissue.

---

[3] *Ibid.* (1906), 46, 1247.

[4] *Lancet* (1904), 2, 1477.

I may quote a few of the most important observations of others in taking up somewhat more in detail the question of symbiosis between bacteria.

Ludwig Hektoen has stated as follows: "It is conceivable that in some cases the combined action of two microörganisms may be necessary to cause a disease. The nontoxic products of two may synthesize to form a toxic substance."

Richie,[5] in his discussion of the general pathology of infection, remarks that "A study of the correlation and interaction between the various organisms frequently or almost invariably found together, may in the future explain many clinical phenomena and observations as yet obscure." And again the same author writes as follows: "The metabolic *products of certain, it may be harmless, microörganisms are often capable of rendering a nonpathogenic genus pathogenic.*"

It is generally accepted that many of the bacteria normally found in the intestine may under proper surroundings produce disease. Symbiosis is undoubtedly one factor in these changes. Vallagussa found that colon bacilli from the intestines of cats were more virulent when a vegetable rather than a meat diet was given to the animals and that the same bacilli were more virulent when taken from decomposing fæces than when secured directly from the intestine. Coco demonstrated that the virulence of colon bacilli was increased by the presence of streptococci and staphylococci, for mixed cultures caused fever when introduced into the intestines of animals, while pure cultures of either did not do so.

Atlasoff[6] found that *B. typhosus* grew better in acid media in symbiosis with *Torula rosæ* and that when these symbiotic organisms were fed together to young rabbits, a fairly typical typhoid disease was produced.

M. Neisser cultivated on blood agar, colonies of influenza and xerosis bacilli from a case of conjunctivitis caused by measles. He continued the cultivation of the mixed bacteria on ordinary agar, and was successful for twenty generations with an organism which, before his work, had never passed beyond two generations. The influenza bacillus would not grow on ordinary agar without *B. xerosis*. When dead xerosis bacilli were used, the cultures failed and this fact proved that it is not the body of the *B. xerosis* which aids the growth of the other organism, but rather its fermentative action on the medium. Klein[7] showed that bacilli of the Gaertner group had an enhancing value on the virulence of *B. typhosus* when they were grown together with the latter in culture tubes.

---

[5] Albutt's System Med. (1907), **2**, pt. 1, 1–198.

[6] *Ann. Inst. Pasteur*, **18**, 701.

The mechanism by which the facultative aërobic spirillum of cholera, acting in the anaërobic environment of the intestine, produces such intense toxæmia has not been satisfactorily explained and the influence which symbiotic bacteria may exert here needs to be investigated. Several years ago Nägeli advanced the hypothesis that cholera was the result of the coöperative action of two kinds of bacteria. Experimenting along this line Metchnikoff found a bacterium which greatly increasd the pathogenic action of *S. choleræ* and Nencki has described bacteria which in themselves were nonvirulent, but which added greatly to the virulence of *S. choleræ* when they were grown together. Musgrave and Clegg have maintained the virulence of a strain of *S. choleræ* for more than one year by growing it symbiotically with amœbæ. The same strain grown pure on artificial media lost much of its virulence during this time.

There is abundant evidence to show that the symbiosis of the diphtheria bacilli and of the streptococci enhance the virulence of both organisms in many cases of diphtheria complicated by streptococcus infection.

There are also certain bacteria and products of bacteria which are antagonistic to other bacteria or diseases. Emmerich, Low[8] and others have fully established an active antagonism of the products of *B. pyocyaneous* for the anthrax bacillus and for several other organisms. In a somewhat similar manner Rettger[9] and others have proved an antagonistic action of the products of *B. prodigiosus* for certain other bacteria. Turro[10] found that subcutaneous injections of beer yeast would protect rabbits aginst otherwise fatal inoculations of streptococci and staphylococci. This work has also been confirmed and amplified by other observers. Lode[11] cultivated a diplococcus which possessed marked antagonistic properties toward several well-known bacteria, among them staphylococci, *B. anthracis, B. cholera susis, B. typhosus* and the cholera spirillum. This diplococcus not only had the power of destroying these bacteria in cultures, but this property was also present in Berkefeld filtrates of the cultures.

---

[7] *Lancet* (1904), **2**, 477.

[8] Emmerich and Low: *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1899), **31**, 1; *ibid.* (1901), **36**, 9. Emmerich: *Centrbl. f. Bakteriol. Orig.* (1902), **32**, 82. Low and Korschun: *Ibid.* (1902), **31**, 1. Emmerich and Trommsdorff: *Ibid.* (1903), **33**, 627.

[9] *J. Infect. Dis.* (1905), **2**, 562.

[10] *Centrbl. f. Bakteriol. Orig.* (1903), **34**, 22.

[11] *Ibid.* (1903). **33**, 196.

Conradi and Kurpjuweit[12] showed that the growth of *B. typhosus* and *B. paratyphosus* was definitely inhibited by *B. coli*. The inhibiting and antagonistic action of *B. coli* toward some other organisms has been shown by a number of authors.

Gabricewski and Maljatai[13] found that *S. choleræ* inhibited the growth of *B. coli*.

Heineman[14] found that streptococci isolated from milk interfered with the development of *B. lactis aërogenes*.

Herter[15] has demonstrated repeatedly that streptococci grown from the human intestine may repress cultures of *B. coli* from the same source.

Walker showed that the typhoid bacillus when grown in the presence of its antiserum acquires a greater virulence for animals and that a larger dose of protective serum is necessary to save guinea pigs from infection with the immunized culture than is needed to protect from the same strain which had not been immunized. Wechsberg demonstrated that diphtheria bacilli could be made to produce a larger amount of toxin by adding diphtheria antitoxin to the culture media in which they are grown.

Many more examples of the above conditions might be mentioned, but these suffice to show the influence which surroundings, even in the test tube, may have upon cultures of bacteria. This influence might properly be considered under the headings of *nutrition, metabolism* and *production,* and the relation between these three conditions is surely more close and significant than we are accustomed to consider it to be in laboratory technique. If such influences as these may modify our artificial cultures of bacteria, how much greater may be the effect upon the three factors mentioned above of the more complex conditions surrounding mixed cultures in unknown symbiosis. Furthermore, what a mass of additional factors are brought forward when we attempt to study the results of the three we have considered, acting and reacting between such a complex of organisms and the animal tissues.

Vaughan, in studying the chemistry of bacterial cells, found that so many definite bodies could be split off along fixed lines of cleavage that he concluded the bacterial cell to be made up of a compound of various chemical groups. If this hypothesis be true it seems reasonable to believe that the character of the various chemical groups would depend partially, at least, upon the character of the substances which may be brought together in the environment.

Ricketts, in discussing the hypothesis of Welch, says: "Certain constituents of our body fluids may, by combining with suitable bacterial receptors, stimulate the bacteria to the production of a whole shower of cytotoxins * * * the nature of the animal substances * * * is not of essential importance * * *. It is not necessary that they be toxic for the bacterium and they may even be taken up as food substances."

---

[12] *München. med. Wchnsch.* (1905), 52, 2164, 2228.

[13] *Centrbl. f. Bakteriol.* (1893), 13, 780.

[14] *J. Infect. Dis.* (1906), 3, 173.

[15] Bacterial Infections of the Digestive Tract. Macmillan, 1907.

This quotation, again, at least in part, discusses a condition of production, metabolism and nutrition. The requirements of a certain form of production calls for an alteration in metabolism, which in turn may demand a modification in nutrition. The ability to answer the call for production, and therefore a determination of the progress of pathologic lesions, must depend to a certain extent on the ability of the symbiosis to supply the required nutrition.

ANIMAL AND BACTERIAL SYMBIOSIS.

Nutrition, metabolism and production in animal parasites certainly are more complicated than with bacteria, and unless we bear this fact constantly in mind the whole subject of parasitism is apt to be considered along too narrow lines and our specificities to be regarded as too exacting.

The question, just as with bacteria, not only involves a study of the interaction between an animal host and animal parasite, but a study of host under constantly varying conditions being acted upon by parasites influenced by an ever-changing environment.

A battle, as it were, takes place between two sets of very complex influences, leading to death or disease of the host on the one hand, to destruction or commensalism on the part of the parasite on the other, or finally quite frequently to what Theobald Smith has called a condition of balanced parasitism.

Very little has been done bearing directly upon the subject of this interesting question of the influence of symbiosis upon animal parasites and most of the literature available concerns itself with amœbæ. The association of certain animal parasites and bacteria in both saprophytic existence and in pathologic lesions was formerly generally considered to be an accidental one, but the idea that the relations between these various types and combinations are of a definite nature and that such associations exercised a specific influence upon the nature and production of disease processes is of comparatively recent origin, so that the amount of evidence is still distressingly small.

When we closely consider this question we find support for the positive side in the suggestions of many careful observers and in all experimental work with amœbæ. Baumgarten (1890) suggested the probable coöperation between amœbæ and bacteria in the production of dysentery. Janowski (1897) considered it not improbable that the symbiotic organisms may determine the pathogenicity of the amœbæ themselves, and that the symbiosis found in nature may be carried unbroken into the intestine, or that it may be formed in the bowel. Such observations have now been repeated and amplified by so many authors that they have appeared in some of the modern literature as proved facts.

Since 1904 Musgrave and Clegg have been publishing the results of researches bearing directly upon this subject. It was first brought forcibly to our attention in the cultivation of amœbæ, which we could not accomplish in a satisfactory manner except in the presence of other living microörganisms, nor has a larger experience and maintained interest given any different results. From the first, the association of bacteria and amœbæ appeared to be very intimate and subsequent work which has been published and need not be reviewed here, has demonstrated some such association to be necessary for the propagation of amœbæ under any circumstances but those of pure parasitism. As a result of more than four years of this continuous work the following suggestions are offered:

1. Amœbæ in any natural environment, whether outside or within the body (excepting in certain liver abscesses and a few other situations), lead a parasitic life in that they feed upon, or in some other way sustain life by the presence and often if not usually at the expense of other living microörganisms.

2. This symbiosis, even when the parasites are in a very mixed bacterial environment, is more or less specific for certain elements of this environment.

3. This specific character of the symbiosis may be changed in artificial media and the evidence points to the occurrence of such changes in nature.

4. All the parasites present in any given environment—the intestine included—are not in symbiosis with the same bacterium.

5. If amœbæ in symbiosis with a given bacterium, after introduction into the intestines of animals, succeed in remaining and propagating there, it is usually by a change of symbiosis to an organism which was already present in the intestine.

6. All amœbæ which reach the intestines of animals in a viable condition, whether as spores or as encysted or vegetative parasites, probably do so either in an active symbiosis, or in a receptive condition for some more or less special symbiosis. The question of proliferation in the intestine probably depends—at least at first—upon the maintenance for a time of the old symbiosis or the ability to change to another one with living microörganisms present in the bowel.

7. During this primary stage of intestinal infection and so long as the true bacterial symbiosis in maintained, it is probable that no particular harm is done to the bowel, regardless of the source or variety of amœbæ present. However, as soon as the tissue components or secretions begin to influence the metabolism or effect other changes in this bacterial-amœbic compound, it is not unreasonable to believe that return manifestation toward the tissues may become a property of the amœbic-bacterial symbiosis, and that lesions of the bowel result.

8. From this amœbic-bacterial-intestinal compound lesion some process may be evolved changing the metabolism of the parasite and lowering the tissue resistance until a focus of infection in which the bacteria have been eliminated, is established in the liver, brain, spleen or some other organ, and thus a lesion is produced which is due to an interaction between the amœba and the animal tissues, a condition of true animal tissue-parasitism.

9. In considering the occurrence of true parasitism we must never lose sight of the fact that the amœba is altered as well as the tissue, which by change has become capable of being the host of the microörganism.

Among the higher animal tissue-parasites in which bacteria play no part in the symbiosis, we still have modifications due to environment, just as are shown by pure cultures of bacteria.

Lingard,[16] showed that the virulence of *Trypanosoma evansi* is to a certain extent influenced by conditions which affect the pathogenicity of bacteria. This author passed an organism of surra directly from horse to horse through forty animals. During the first part of the experiment the incubation period varied from four and one-half to six and one-fourth days and the duration of illness from twenty-five to forty-two days, but during the latter period the incubation time was reduced to from three to four and one-half days, and the duration of the disease to from three to four days. The author made the further interesting observation that white mice inoculated from a horse in the early stage of his experiments showed an incubation period of from five to five and one-half days and a duration of the disease of five days. The infection was then transferred from mouse to mouse, and between the thirty-seventh to forty-seventh mouse instances occurred in which the incubation was reduced to twelve hours and the course of the disease to from two and one-half to three days. Still more interesting is the fact that a horse inoculated from the forty-seventh mouse lived four and one-half times as long as the control animal.

Some of the influences which a change of host may exert on parasites are well illustrated in smallpox. As is known, the pustular contents of smallpox when inoculated into monkeys produces a disease which clinically may closely resemble variola in man. When some other of the lower animals, such as the cow, are used for these experiments, the disease reproduced in the animal differs materially in its manifestations and when the virus is again transferred from the cow to man it manifests itself as vaccinia, which differs from smallpox, and human variola can not be reproduced from this virus.

Satisfactory explanation of this phenomenon has not been furnished beyond the obvious fact that in some manner the pathogenic character of the organism is changed. Great variations in the pathogenic nature of the cause of smallpox, which are due to other influences, are shown in the marked difference in the severity of the disease in various epidemics. Not infrequently the disease in certain outbreaks may appear to be so mild as fairly to justify the statement that the infection is harmless, and again under apparently similar conditions or during the same epidemic, the reverse condition of severe infection and high mortality may obtain. Such changes are probably not due to a difference in the condition of the host as is the case in vaccinia, but to some other symbiotic or environmental influence acting with or upon the parasite, and as Professor Councilman has pointed out, the part played by the constantly present symbiotic bacteria in these lesions has not yet been determined, but it is probably an important one.

---

[16] Annual Report, Imp. Bact. of India (1906-7).

CONCLUSIONS.

It seems to me that the evidence is fairly conclusive in favor of a more or less definite symbiosis, for example between amœbæ and other microörganisms, whether they be found in a saprophytic existence or in pathologic lesions, with the exception of the true animal parasitism found in certain bacteria-free abscesses in the animal host, and this parasitism is evolutionary in character.

In other words, a condition of parasitism exists even under outside conditions, a parasitism between bacteria and amœbæ which is not only a changeable one, but which may be evolutionary, gradually passing from the extraneous surroundings to be found in water and other saphrophytic environment, through the mixed infections encountered for example in dysenteric ulcers, where the elements of nutrition of the amœbæ may consist of animal secretions or tissues, modified or reënforced by the bacteria, to a condition of true animal tissue-parasitism found in certain abscesses of the liver and other places in the body, or even to the still more exquisite parasitism of amœbæmia, two cases of which have been observed by Mr. Clegg and myself.

There does not appear to be anything particuluarly radical in such a hypothesis, especially when one considers what has been proved about the action of mixed cultures of bacteria in respect to symbiosis and when it is remembered that at least that part of this evolution of parasitism in which amœbæ pass from the mixed environment of the intestinal lesion to a more specific tissue parasitism is a matter of routine observation in the tropics.

I am convinced, as has been stated above, that such an evolution of parasitism from an amœbic and bacterial symbiosis in water or elsewhere in nature, through a beginning or mixed tissue and bacterial parasitism in amœbic ulcers of the colon, to a true tissue parasitism in the internal organs of the body, or even to a blood invasion itself, is a matter of frequent occurrence. However, the extent or generality of such a process and the elements necessary for its beginning and propagation and the influences which antagonize or favor it are only slightly studied.

The whole question is of the most intense interest and of the greatest practical importance and offers one of the most promising fields for research to be found at the present time. It is not inconceivable that the whole process of parasitism, even in its greatest selectiveness, as for example of tetanus toxin for the nervous system or of certain trypanosomata for cerebro-spinal fluid, is evolutionary and there is not lacking evidence which may be used in support of such a hypothesis with more logic than has hitherto been given in attempted explanation of many phenomena.

The most promising field for laboratory research in the future will be the study of cause and effect, in the complex relations in which they occur in nature, of the interrelation and interaction of microörganisms with each other and in their environment of complex symbiosis and the ever-changing and multiple conditions found in hosts. Attention to individual components of microörganisms and specific manifestation of resistance by hosts without being accompanied by a balanced conception of the influence of complex environment upon the details, has undoubtedly led to the elaboration of a considerable superstructure which is useless if not dangerous in the study of pathology.

SOME CONSIDERATIONS WITH REGARD TO THE CAUSE OF THE FREQUENT REAPPEARANCE OF CHOLERA IN THE PHILIPPINE ISLANDS, WITH STATISTICS BEGINNING WITH THE OUTBREAK IN 1902 TO JANUARY 1, 1908.[1]

By Victor G. Heiser.

(Director of Health and Chief Quarantine Officer for the Philippine Islands, passed assistant surgeon, U. S. Public Health and Marine-Hospital Service, professor of hygiene, Philippine Medical School.)

INTRODUCTION.

The Bureau of Health has been called upon to combat cholera more or less continuously from the time of the publication of the paper which was read upon the subject of cholera before the Manila Medical Society January, 1906,[2] up to the present. The total number of cases and deaths reported from the beginning of the outbreak in 1902 to January 1, 1908, was as follows:

Table I.—*Cases and deaths from cholera in the Philippine Islands from the beginning of the outbreak in 1902 to January 1, 1908.*

Date.	Cases.	Deaths.
March 2, 1902, to March 8, 1904	166,252	109,461
March 8, 1904, to January 1, 1908	11,691	8,654
Total	177,943	118,115

Mortality, 74.03 per cent.

It is apparent from the foregoing figures that cholera has caused great havoc in these Islands. This is greatly to be deplored not only from the standpoint of the useless loss of human life, but also because such conditions retard the development of the country by reducing the already too small number of workers.

At first sight it would appear as if the diffusion among the masses of the plain, simple truth, that cholera can only be contracted by the introduction into the mouth of contaminated food or drink, and that almost absolute safety against infection can be insured by the simple precaution of using sterilized water and cooked food, would result in its early disappearance, but when it is remembered that the climate is favorable for the development of the cholera organism throughout the four seasons of the year—in other words, that there is no winter season which would at least stop the disease for a time—that a large portion of the people are ignorant and inaccessible, that much superstition exists, that one of the most popular beliefs is in the supposed injurious character of boiled water, that the cost of fuel is comparatively high, making sterile water and cooked food difficult for the masses to obtain, that the majority of the people cling tenaciously to the mode of living which has been customary with them for hundreds of years, that food is conveyed to the mouth with the fingers from a receptacle used by all the household, that over 60 per cent of the population are afflicted with intestinal parasites, that, with the possible exception of the supply for a few hundred thousand out of a total of seven million, the drinking water is obtained from shallow surface wells, that physically the people are weak and unresistant, and that funds and skilled physicians needed to combat this condition are very limited, it will not be so difficult to understand the continued presence of cholera in the Philippines. If better results or the complete eradication of the diseases are to be hoped for, additional methods beyond those which have heretofore been used in any part of the world will need to be made available. In order that such methods may be determined upon in a scientific manner, it will be necessary to make further advances in the study of the cholera organism, both from a morphological and epidemiological standpoint. The following deductions based upon observations of the cholera during the past five years are submitted with the hope that some progress may be made in this direction.

---

[1] Read at the Fifth Annual Meeting of the Philippine Islands Medical Association, February 28, 1908.

[2] *J. Am. Med. Ass.* (1907), **48**, 856.

PAST EPIDEMICS IN THE PHILIPPINE ISLANDS.

It will perhaps be recalled that the first case of cholera, recognized as such since American occupation, occurred in March, 1902. It is difficult to determine satisfactorily whether any cases of the disease had occurred between the epidemic of 1888 and of March, 1902. This is a question, however, which has given rise to considerable discussion. Physicians who were resident in the Philippine Islands prior to American occupation claim that cholera has been continually present for many years, but it is generally held by Americans that because no record exists of a microscopic confirmation of such cases, the diagnoses are open to doubt. In view of the foregoing, it is of course evident that this question can never be definitely and satisfactorily settled. Certain it is, however, that between 1897 and 1902 there is no record of any other disease in the Philippine Islands which shows such a large mortality, nor was any such infection present in so many portions of the Islands as has been the case since 1902. The commencement of the disease in 1902 was ascribed to a shipment of presumably infected Canton cabbage arriving in Manila from Hongkong which upon being refused landing, was thrown overboard in the harbor, with the result that the whole bay was literally covered with this vegetable. Much of the cabbage was gathered and consumed and within forty-eight hours from the time of its reaching the beach, cases of cholera commenced to occur in the Farola district[3] (see Pls. I and II), which is situated at the junction of the Pasig River and Manila Bay. An Army transport which left Manila at about the same time these vegetables were thrown overboard, proceeded directly to Nueva Caceres and the disease was discovered among some steerage passengers of this ship after they had landed, so that cholera appeared simultaneously in two widely separated places in the Philippine Islands.

The infection spread rapidly in Manila in spite of the most energetic measures which were taken to combat it. The cordon which was placed around the city was entirely ineffective and apparently offered little resistance to the onward march of cholera. Infected persons gradually introduced the disease into the other islands. The outgoing maritime quarantine imposed upon vessels was ineffective because people traveled overland to ports at which there was no adequate quarantine inspection, and sailed from there. Cholera raged with great severity for nearly six months in and about Manila (over 6,000 cases occurring in this city alone) and it continued for about an equal length of time in other portions of the Islands, after the date of its first appearance. There was a rather severe recrudescence during the following year in Iloilo and Cebu. The Board of Health for the Philippine Islands on April 27, 1904, officially declared the disease to have been completely arrested, and stated that no cholera was then in the Islands.

The next appearance in recognizable form took place in August, 1905, when persons in the neighborhood of Jala Jala, in the Province of Rizal, on Laguna de Bay (see Map No. 1), commenced to die from the disease, and from there it apparently spread rapidly down the Pasig River to Manila. Since 1905 the disease has been practically confined to Manila and the provinces which are accessible by land from this city. The only exceptions to this rule are the outbreaks which occurred in the Provinces of Iloilo, Occidental Negros, northern Samar, Mindanao, and Leyte.

---

[3] The Farola district is a narrow neck of land running out into the sea and having the main harbor light at its point. It was occupied at the time of the outbreak of this epidemic by warehouses, coal piles and a crowded mass of small, insanitary huts.

CHOLERA-LIKE CASES PRECEDING TRUE OUTBREAKS.

It is noteworthy that between March, 1904, and August, 1905, cases resembling cholera were encountered every few weeks in and about Manila. These died with practically all the clinical symptoms of cholera, but upon bacteriological examination, cholera vibrios could not be demonstrated in the stools or at autopsy. This fact has received considerable attention and since 1905 it can definitely be stated that the last three outbreaks in Manila, namely that of August, 1905; of June, 1906, and of August, 1907, were invariably preceded by suspicious deaths of the kind mentioned above. Cases suspicious of cholera occurred at intervals of one to four weeks in various sections of the city of Manila as early as April, 1905.[4] Possibly, in all, from 10 to 15 deaths of this kind in which a positive microscopical diagnosis could not be made came under observation before each outbreak. In view of the fact that all deaths in the city of Manila are investigated before a burial permit is issued, it is not likely that many of these cases escaped observation.

Even after the disease establishes itself it rarely happens that two infections occur in any one house or in a group of houses, or even in the same block. The isolation of the case and the prompt disinfection which is made of the premises in each case may account for this fact in part.

OTHER OBSERVATIONS IN REGARD TO THE EPIDEMIOLOGY.

Many isolated observations, which seemed in some way to be intimately connected with the spread of the disease were made since 1903. It has been noted that when flies are particularly active and persistent and refuse to be driven away, as, for instance, is the case in the United States before a thunderstorm, or when large numbers of roaches, water bugs, and other vermin were seen to be moving about actively in the streets, or when the humidity is relatively high, or bright sunshine is absent with only a small amount of rain, immediately a considerable increase in the number of cholera cases almost invariably follows. In 1903 it was observed that the still water in the bends of the Pasig River was infected with cholera organisms, while the main current of the river remained uncontaminated.

The outbreak of the disease, for instance in Manila, practically always commences among the lowest members of society and frequently beggars or other persons who eke out a precarious existence are the first to be attacked. An examination of the order in which cases appeared in the city of Manila during 1905 and a comparison with the subsequent outbreaks shows that the same condition of affairs which existed then has been present ever since, namely, one case will occur in Tondo district, the next possibly in Malate, the next perhaps in Sampaloc, all generally a mile or more apart, and the most careful investigation fails to reveal any connection whatsoever between the infections. After this beginning the disease seems to increase in virulence and gradually commences to attack persons who are better nourished and fed, until finally more resistant persons like Europeans, the better class of Filipinos, and Americans become victims.

---

[4] *Loc. cit.*

It has been most difficult to explain the presence of cholera in Bilibid Prison each time that it makes its reappearance in the city of Manila. In this institution sterilized water is supposed to be invariably furnished and only cooked foods are served and every reasonable precaution is taken. The outbreaks in this institution have heretofore never been satisfactorily traced or accounted for.

The practical observation made several years ago that an outbreak of cholera is never severe in a community which was afflicted within two years has been of great assistance in combating this disease. Another noteworthy fact is that a much larger percentage of cases occurs among the Japanese than among any other nationality, and this is particularly true of the Japanese fishermen who leave the shore every evening at sundown and spend the night upon the bay in fishing; returning again about daylight. These men are usually attacked during the latter portion of the night.

The appearance of the disease in the provinces which are accessible from Manila by land apparently is never preceded by the suspicious deaths which have been observed in this city, and so far there has been very little difficulty in tracing such outbreaks to a previous case. On the other hand, those which have occurred in the Provinces of Iloilo, Samar, and Leyte (see Map No. 2), which are separated from Manila by the open sea, have invariably begun among the poorest members of the community and the first cases could not be traced to a previously infected person.

FACTORS SUGGESTING HYPOTHESIS.

During the past five years in order that improved methods for stamping out cholera might be devised, every endeavor has been made to formulate a hypothesis which would explain the appearance of this disease under such unusual circumstances, but up to within a few weeks ago this has been impossible. In consequence, it has not been practicable completely to eradicate the cholera from the Islands by employing the usual sanitary measures. Of course, it is well known that if all the residents of a community could be induced to drink sterile water and eat cooked foods the disappearance of cholera invariably wuld take place, but with a population like that of the Philippine Islands, it will be obvious to those who have had experience with local conditions that many years of education would be required before a result of this kind could be hoped for; so that if greater success is to be expected, a more direct method of attacking the problem will have to be devised. The fact that the disease seems to reappear in the city of Manila in a form which clinically resembles cholera, apparently offers a key to the problem, especially as it is coupled with the other fact that the study of the organism at the Biological Laboratory of the Bureau of Science by different workers shows that considerable change in its morphology takes place. Clinically, such cases give a history of sudden onset with vomiting which lasts for a period of six to twenty hours, marked diarrhœa with stools not typical of cholera, extreme collapse, severe cramps in the arms and legs, profuse sweats, subnormal temperature, rapid pulse, anuria, and other evidences of intense intoxication, for which active stimulation, external heat, hypodermoclysis, intravenous transfusion of salt solution, or serum are of no avail. On post-mortem these cases show marked rigor; dryness of the abdominal cavity; intestinal contents not typical of cholera; the ileum and large intestine studded with small, bead-like elevations; evidences of intense acute nephritis and punctiform hemorrhages upon the serous surfaces of the heart, and cultures made by such competent observers as the chief of the Biological Laboratory and his assistants fail to reveal cholera organisms. However, each succeeding case gradually approaches more and more the findings of cholera both clinically and at autopsy, until finally the autopsies are typical and the cholera organism is recovered from cultures taken from the intestines. When this stage is reached, the disease also spreads much more rapidly throughout the city, the number sometimes reaching as high as 70 cases per week, with a mortality of 90 per cent; this then gradually declines, and the mortality drops to 40 or 50 per cent. An outbreak of this kind extends over a period of from two to three months. After the organisms once become recognizable, there is no difficulty in identifying them until the disease completely disappears, although there is considerable change in their morphology, which will be shown in a subsequent paper by H. T. Marshall.

The fact that the cause of the outbreak of cholera in the provinces of the Philippines seems invariably to be traced to Manila, the disease either being transmitted by the infected persons or foods, and since the first cases in Manila after 1904 are not traceable to any outside source, it is evident that some conditions must exist in this city favorable to the continuance of the cholera organism which do not obtain in other portions of the Islands. It does not appear probable that the organism remains in a quiescent stage in the intestines of the residents, because in that event there certainly would be many outbreaks in other portions of the Islands of the same heretofore inexplainable kind as these which have occurred in Manila. It seems certain, to judge from our knowledge of the cholera organism, that it could not exist in a dry state and since a constant wet medium is indispensable, it appears probable that the old sewer system of the city of Manila offers one of the few conditions in which the latter is assured during every day in the year. It is scarcely conceivable that a pathologic cholera vibrio is propagated in the city water supply because a much larger number of cases would surely occur.

HYPOTHESIS.

With the conditions then as set forth in the foregoing pages, the following hypothesis is submitted: The cholera organism is continuously present in some form in the sewer system of Manila either in its fluid contents or in vermin; at certain stages of its atypical form, by passage through the human intestine or otherwise, it first is capable of producing a disease resembling cholera, but the organism can not be recognized by the present laboratory methods. Successive passages through the human intestine produce a typical cholera vibrio. Possibly the disease disappears because the population gradually becomes immune by eating fish and other sea products which have been taken from cholera-infected waters, or drinking the water itself. An immunity of this kind appears probable from the fact that cholera organisms continuously passed through the living, susceptible intestines either increase or retain their virulence, and this should cause the mortality among those attacked throughout an epidemic to remain the same. However, the reverse is the case, as may be seen by referring to the history of the cholera outbreaks in the Philippines and other countries (the rate at the beginning being from 90 to 100 per cent and soon falling to 40 per cent or below), so that the decline in the mortality would seem to be due rather to the acquired resistance of the individual than to the change in the cholera vibrio.

CONDITIONS CONFIRMING THE HYPOTHESIS.

It appears probable that the cholera organism is conveyed either directly or indirectly from the sewers to easily contaminated articles of food or drink by the means of roaches, either through direct mechanical contact with their feet or bodies, or by the deposition of their excretions, thence it is passed to human beings; but flies, water bugs, and other vermin commonly found in sewers are also no doubt partly responsible.

When it is remembered that cholera organisms can live in the intestines of such vermin for a number of weeks, it will be at once apparent that many cases of cholera, the origin of which has heretofore been obscure, will have at least a reasonable explanation. It has frequently happened that only one case occurred in a house in which seventy-five persons or more obtained their meals and the fact that rarely ever more than one case occurs in a city block shows a limited infection which can be explained by insect transmission. An outbreak of cholera such as occurred in Honolulu during the latter part of December, 1907, might also be explained by the landing of infected roaches.

As soon as proper health measures are put into effect to provide for the disinfection of the stools of infected persons and the community gradually acquires a temporary immunity, the outbreak slowly subsides, and the disease does not again make its appearance in Manila until it is either introduced from the outside or the cycle given above is repeated.

It is conceivable that the immunity which has been referred to is due to drinking city water, which at times contains a vibrio similar to that of cholera but this does not appear probable. The outbreaks in the provinces which are separated from Manila by open sea seem to be caused by infected food, and this seems especially true if we consider the observation made by Dr. Cullen that the first persons attacked last yaer in northern Samar all had eaten a poor quality of dried fish taken from waters in Manila that were presumably infected.

The fishing industry in and about Manila is conducted on a most extensive scale. The low, marshy character of the section of the bay north of the Pasig River and the rise and fall of the tide, produce ideal conditions for the growth and contamination of shell and other fish, and this is especially accentuated since the tidal currents are such that the contents of one of the city's largest sewers is extensively distributed throughout this area. Granting, then, for the sake of argument, that fish, shellfish, and other sea products become infected with cholera and that through imperfect cooking the organisms are not all killed, and that one of the principal food substances of the masses in the city of Manila is gathered from the section just mentioned, it is evident that it would be possible to immunize a large portion of the city's inhabitants. Enormous quantities of sea products in a dry or partially dry state are also sent to various portions of the Islands from Manila and if it should happen that some of them have been shipped in a moist condition or are otherwise rendered a good culture medium of cholera vibrio, it is conceivable that such products might, in isolated instances, be the cause of appearances of cholera in places such as Samar, Leyte, etc. In view of the fact that this class of food products is the sustenance of the masses because of its cheapness, it is more probable that the underfed members of a community would be the first to be affected by the slight infection which it might contain.

In order to test the foregoing hypothesis, the Bureau of Science has undertaken a laboratory study of the matter, and already investigations made within the past few weeks of the sewers of the city of Manila show that the cholera organism was present in them, and that almost simultaneously with the disappearance of the disease in human beings, about two weeks ago, it has been impossible to find any cholera vibrios in these sewers. In view of the results of this study, it appears probable that the cholera vibrio may live in the old Spanish sewers of the city of Manila indefinitely. This fact may not be capable of demonstration the year around, but the failure to find the organism is due to the change in its morphology. It also appears probable that at such times as the organism can not be recognized as the cholera vibrio, there is a stage during which it is capable of producing cases resembling cholera, such as have been noted in each instance before an actual outbreak.

CONCLUSION.

In conclusion, then, it may be justifiable to state that, it appears highly probable, cholera is endemic in the city of Manila; that the habitat of the organism is in the sewers of the city; that outbreaks in the Philippines outside of Manila are probably due to direct infection from the city of Manila by the food products which are sent out from here, or by direct infection by the means of persons carrying the disease. The cases which occurred in the provinces which can be reached by land outside of Manila are in nearly every instance caused by direct infection by means of human beings, and not from food products. This peculiarity is probably due to the fact that very little food is sent from Manila to near-by towns, because they have similar supplies within their own borders.

Briefly, then, it is believed that if the sewer system of Manila could be throughly disinfected and rendered free of the cholera organism in any of its forms, and the egress of vermin could be stopped, one of the principal sources of the disease would be removed and the early disappearance of the infection from the Islands could be hoped for.

The following tables demonstrate that the average duration of cholera in a town is about two months.

TABLE IV.—*Cholera statistics arranged in the order in which the towns became infected.*

JANUARY TO JULY, 1906.

Date of first case.	Town.	Province.	Highest number of cases.	Date of last case.	Total number of cases.
Jan. 3	Naic	Cavite	Jan. 24	Feb. 26	195
Jan. 8	Macabebe	Pampanga	May 21	June 28	97
Jan. 4	Abucay	Bataan	Jan. 16	Mar. 8	66
Jan. 4	Bulacan	Bulacan	Feb. 15	Mar. 2	19
Jan. 4	Noveleta	Cavite	Jan. 31	Feb. 3	58
Jan. 4	Calumpit	Bulacan	Jan. 15	June 29	65
Jan. 4	Malolos	do	Jan. 4	June 30	41
Jan. 4	Quingua	do	Jan. 11	June 30	88
Jan. 4	Imus	Cavite	Mar. 24	Apr. 5	36
Jan. 5	Hagonoy	Bulacan	Jan. 5	Mar. 8	33
Jan. 5	Lilio	La Laguna	Jan. 5	Jan. 5	2
Jan. 5	Magdalena	do	Jan. 5	Jan. 15	3
Jan. 5	Majayjay	do	Jan. 5	Jan. 15	2
Jan. 5	Pacte	do	Jan. 12	Mar. 7	36
Jan. 5	Guagua	Pampanga	Jan. 17	Feb. 5	50
Jan. 5	San Luis	do	June 16	June 30	120

TABLE IV.—*Cholera statistics arranged in the order in which the towns became infected*—Continued.

JANUARY TO JULY, 1906—Continued.

Date of first case.	Town.	Province.	Highest number of cases.	Date of last case.	Total number of cases.
Jan. 5	Malabon	Rizal	Jan. 24	Feb. 1	27
Jan. 6	Tarlac	Tarlac	Jan. 30	Feb. 1	8
Jan. 8	San Roque	Cavite	Jan. 8	Jan. 8	1
Jan. 8	Santa Cruz	La Laguna	Jan. 12	June 30	15
Jan. 10	Balanga	Bataan	Apr. 12	Apr. 19	88
Jan. 11	Paombong	Bulacan	Jan. 11	Feb. 26	48
Jan. 11	Cabanatuan	Nueva Ecija	Jan. 11	Feb. 28	42
Jan. 12	Pila	La Laguna	Mar. 7	Mar. 7	2
Jan. 12	Baliuag	Bulacan	Jan. 31	Apr. 18	70
Jan. 12	Lubao	Pampanga	Jan. 25	May 24	56
Jan. 13	San Francisco de Malabon	Cavite	Feb. 2	Feb. 23	57
Jan. 13	Cavite	do	Jan. 26	June 30	20
Jan. 15	Nagcarlang	La Laguna	Jan. 15	Jan. 15	1
Jan. 16	Orion	Bataan	Feb. 21	Apr. 19	84
Jan. 16	Pagsanjan	La Laguna	Jan. 22	June 30	10
Jan. 16	Arayat	Pampanga	Apr. 26	Apr. 28	108
Jan. 16	San Fernando	do	Jan. 19	Mar. 31	5
Jan. 17	Bongabong	Nueva Ecija	Jan. 17	Jan. 17	1
Jan. 18	Stotsenberg	Pampanga	June 13	June 13	2
Jan. 19	Candaba	do	Feb. 5	Mar. 10	49
Jan. 22	Angat	Bulacan	Jan. 27	Mar. 2	127
Jan. 22	Biñang	La Laguna	Jan. 22	Jan. 22	1
Jan. 22	Gapang	Nueva Ecija	Jan. 22	Feb. 20	36
Jan. 22	Apalit	Pampanga	Jan. 22	Feb. 27	59
Jan. 22	Calamba	La Laguna	Jan. 22	June 30	3
Jan. 25	Santa Maria	Bulacan	Jan. 27	Mar. 7	39
Jan. 25	Florida	Pampanga	Jan. 25	Jan. 25	1
Jan. 25	Mexico	do	Jan. 25	Mar. 20	2
Jan. 25	Santa Rita	do	Jan. 25	Feb. 26	8
Jan. 26	San Pedro Macati	Rizal	Jan. 26	June 28	8
Jan. 29	Maragondon	Cavite	Feb. 23	Mar. 10	70
Jan. 29	Angeles	Pampanga	Jan. 29	Jan. 29	1
Jan. 29	Bacolor	do	Feb. 20	Feb. 21	4
Jan. 30	Bocaue	Bulacan	Jan. 31	Feb. 20	14
Jan. 30	Indang	Cavite	Feb. 1	Feb. 13	24
Jan. 30	Peñaranda	Nueva Ecija	Jan. 30	Mar. 27	8
Jan. 30	Porac	Pampanga	Jan. 30	Jan. 30	1
Jan. 30	La Paz	Tarlac	Feb. 6	Feb. 10	10
Jan. 31	Orani	Bataan	Feb. 8	Mar. 16	35
Jan. 31	San Antonio	Nueva Ecija	Feb. 13	Mar. 26	7
Feb. 1	Pangil	La Laguna	Feb. 17	Feb. 17	4
Feb. 5	San Isidro	Nueva Ecija	Feb. 16	Feb. 26	8
Feb. 15	Bagac	Bataan	Feb. 15	Feb. 15	1
Feb. 15	Polo	Bulacan	Feb. 15	Feb. 16	3
Feb. 21	Guevara	Tarlac	Feb. 21	Feb. 26	9
Mar. 5	Siniloan	La Laguna	Mar. 5	June 27	142
Mar. 15	Bacoor	Cavite	Apr. 26	May 21	16
Mar. 19	Capas	Tarlac	Mar. 19	Mar. 19	1
Mar. 22	Mabitac	La Laguna	Mar. 22	Mar. 31	2
Apr. 16	Parañaque	Rizal	May 1	May 23	25

TABLE IV.—*Cholera statistics arranged in the order in which the towns became infected*—Continued.

JANUARY TO JULY, 1906—Continued.

Date of first case.	Town.	Province.	Highest number of cases.	Date of last case.	Total number of cases.
May 1	Pasay	Rizal	June 9	June 30	4
May 29	Mariveles	Bataan	May 29	May 29	1
June 9	Pasig	Rizal	June 20	June 30	61
June 12	Antipolo	do	June 12	June 12	1
June 18	Tanay	do	June 23	June 30	145
June 20	San Felipe Neri	do	June 20	June 30	5
June 22	Nampaya	La Laguna	June 22	June 22	2
June 25	Cabuyao	do	June 25	June 29	2
June 26	Taytay	Rizal	June 26	June 28	10
June 28	Taguig	do	June 28	June 28	1
June 29	Bay	La Laguna	June 29	June 29	1
June 30	McKinley	Rizal	June 30	June 30	1

FISCAL YEAR 1906-1907.

Date of first case.	Town.	Province.	Highest number of cases.	Date of last case.	Total number of cases.
1906.			1906.	1906.	
July 2	Pasig	Rizal	July 7	Sept. 9	108
July 2	Fort McKinley	do		Aug. 16	6
July 2	Pasay	do	July 7	Aug. 17	44
July 2	Tanay	do	July 29	Aug. 17	72
July 2	San Felipe	do		July 20	20
July 2	Lumbang	Laguna		July 2	1
July 2	Santa Cruz	La Laguna	July 15	Oct. 31	132
July 2	Pagsanban	do	July 15	Oct. 22	11
July 2	Pila	do	July 4	Aug. 30	49
July 2	Malolos	Bulacan	July 30	Aug. 22	145
July 2	Quingua	do			117
July 2	Cavite	Cavite	Aug. 31	Oct. 26	28
July 3	San Luis	Pampanga			105
July 3	Macabebe	do	July 8	Aug. 18	84
July 3	Mariquina	Rizal		Aug. 3	15
July 4	Hagonoy	Bulacan	July 15	Nov. 23	88
July 5	San Pedro Tunasan	Rizal		July 26	26
July 6	Lilio	La Laguna		July 10	2
July 6	Cabuyao	do	Aug. 16	Sept. 2	38
July 6	Calamba	do		Sept. 4	51
July 7	Bay	do		July 7	1
July 7	San Isidro	Nueva Ecija	Aug. 5	Sept. 3	70
July 7	Taguig	Rizal			43
July 7	Antipolo	do		July 27	3
July 7	Morong	do			22
July 8	Calumpit	Bulacan	Aug. 4	Oct. 1	55
July 8	Bulacan	do	July 15	Oct. 10	40
July 9	Polo	do	July 25	Sept. 22	82
July 9	Angat	do			101
July 9	Santa Rita	Pampanga	July 14		58
July 10	Bacolor	do		Sept. 1	231
July 10	Apalit	do		Sept. 1	55
July 10	Candaba	do			49
July 10	Guagua	do			164

TABLE IV.—*Cholera statistics arranged in the order in which the towns became infected*—Continued.

FISCAL YEAR 1906–1907—Continued.

Date of first case.	Town.	Province.	Highest number of cases.	Date of last case.	Total number of cases.
1906.			1906.	1906.	
July 10	Meycauayan	Bulacan	July 25		30
July 11	Baliuag	do	Aug. 2	Oct. 22	413
July 11	Paombong	do	July 11	Oct. 11	29
July 12	Santa Maria	do			35
July 12	Naic	Cavite	July 19	Oct. 15	63
July 13	Rosario	do	Aug. 22		10
July 13	Pañgil	La Laguna			5
July 13	Malabon	Rizal	July 25	Dec. 28	170
July 13	San Miguel	Bulacan	July 19	Sept. 29	81
July 14	Bocaue	do	July 14	Sept. 26	74
July 14	Angeles	Pampanga			7
July 14	Santa Rosa	La Laguna			54
July 16	Lubao	Pampanga			77
July 16	San Fernando	do			98
July 16	Santo Tomas	do		July 16	1
July 16	Rosales	Pangasinan			4
July 18	Bayambang	do			20
July 18	Imus	Cavite	July 25	Oct. 15	43
July 19	Biñang	La Laguna			22
July 19	Mexico	Pampanga		Nov. 15	17
July 20	Porac	do			17
July 20	Maragondon	Cavite	July 20	Aug. 18	5
July 20	San Jose Guimba	Nueva Ecija		July 20	2
July 21	Cabanatuan	do		Sept. 28	58
July 23	Pagbilao	Tayabas	July 29	Sept. 12	51
July 25	Arayat	Pampanga			58
July 26	Lucena	Tayabas	Aug. 22	Sept. 21	46
July 26	Tarlac	Tarlac	Aug. 5	Aug. 12	14
July 27	Binangonan	Rizal		Aug. 15	20
July 28	Bautista	Pangasinan	Aug. 15	Sept. 10	23
July 29	Peñaranda	Nueva Ecija	Aug. 3	Nov. 13	150
July 29	San Antonio	do	Aug. 4	Sept. 3	20
July 29	Laguimanoc	Tayabas		Aug. 4	7
July 29	Tiaong	do			8
July 30	Floridablanca	Pampanga			16
Aug. 2	Gapan	Nueva Ecija	Aug. 11	Oct. 27	139
Aug. 2	Aliaga	do		Sept. 26	218
Aug. 2	Majayjay	La Laguna			3
Aug. 3	Nagcarlang	do		Sept. 19	66
Aug. 3	Tayabas	Tayabas		Aug. 27	10
Aug. 3	Victoria	Tarlac	Sept. 8	Sept. 25	24
Aug. 3	Caloocan	Rizal			37
Aug. 7	Siniloan	La Laguna			5
Aug. 7	Noveleta	Cavite			27
Aug. 12	Pozorrubio	Pangasinan		Aug. 12	1
Aug. 15	Baguio	Benguet		Aug. 15	1
Aug. 16	Sariaya	Tayabas		Oct. 14	10
Aug. 16	San Francisco de Malabon	Cavite		Sept. 30	4
Aug. 17	Dagupan	Pangasinan			2

TABLE IV.—*Cholera statistics arranged in the order in which the towns became infected*—Continued.

FISCAL YEAR 1906–1907—Continued.

Date of first case.	Town.	Province.	Highest number of cases.	Date of last case.	Total number of cases.
1906.			1906–7.	1906–7.	
Aug. 18	Orion	Bataan			7
Aug. 19	Calasiao	Pangasinan			1
Aug. 25	Pura	Tarlac	Aug. 30	Nov. 8	25
Aug. 25	Talavera	Nueva Ecija	Sept. 16	Oct. 12	108
Aug. 28	Camp Vicars	Moro			9
Sept. 1	Cabatuan	Iloilo	Oct. 2	Oct. 29	366
Sept. 3	Magdalena	La Laguna			5
Sept. 4	Iloilo	Iloilo	Sept. 12	Oct. 21	74
Sept. 11	Moncada	Tarlac		Sept. 12	2
Sept. 14	Binmaley	Pangasinan	Sept. 15		10
Sept. 16	Mabalacat	Pampanga			1
Sept. 18	Dumangas	Iloilo	Sept. 16	Oct. 31	210
Sept. 18	Molo	do			3
Sept. 18	Pavia	do	Sept. 25	Oct. 27	32
Sept. 18	La Paz	do	Sept. 25	Oct. 22	50
Sept. 18	Barotac Nuevo	do		Nov. 2	46
Sept. 19	Jaro	do	Sept. 28	Oct. 13	58
Sept. 21	San Mateo	Rizal		Sept. 21	1
Sept. 21	Nabalas	Iloilo		Sept. 23	6
Sept. 23	Tayug	Pangasinan	Oct. 4	Oct. 15	14
Sept. 24	Los Baños	La Laguna			1
Sept. 25	Igbaras	Iloilo		Sept. 29	2
Sept. 25	Buenavista	do			4
Sept. 27	Miagao	do	Oct. 8	Nov. 8	294
Sept. 29	Guimbal	do			2
Oct. 4	Alimodian	do	Oct. 17	Nov. 5	275
Oct. 8	San Miguel	do	Oct. 17	Nov. 4	195
Oct. 8	Santa Barbara	do		Oct. 27	36
Oct. 8	Leon	do	Oct. 26	Nov. 2	33
Oct. 9	Janiuay	do	Oct. 11	Nov. 16	100
Oct. 9	Banate	do		Oct. 18	12
Oct. 20	Sual	Pangasinan			2
Oct. 27	Camiling	Tarlac	Nov. 3	Nov. 23	26
Oct. 28	Talisay	Occidental Negros		Nov. 19	4
Oct. 28	Silay	do		Dec. 11	7
Nov. 3	Dumarao	Capiz	Nov. 16	Jan. 18	88
Nov. 13	Laguan	Samar		Dec. 22	1
Nov. 13	San Miguel	do			12
Nov. 13	Catubig	do			16
Nov. 22	Dao	Capiz		Jan. 8	15
Nov. 22	Pontevedra	Occidental Negros		Feb. 23	41
Dec. 1	Dumalag	Capiz		Jan. 8	31
Dec. 5	Jamindan	do	Jan. 22	Jan. 26	31
Dec. 8	Palapag	Samar		Mar. 23	42
Dec. 8	Laoang	do			4
Dec. 9	Panay	Capiz			35
Dec. 10	Jinigaran	Occidental Negros		Feb. 28	14
Dec. 23	Pambujao	Samar			3
Dec. 23	Capiz	Capiz		Feb. 16	23
Dec. 27	Tagabiran	Samar		Dec. 27	2

TABLE IV.—*Cholera statistics arranged in the order in which the towns became infected*—Continued.

FISCAL YEAR 1906–1907—Continued.

Date of first case.	Town.	Province.	Highest number of cases.	Date of last case.	Total number of cases.
1907.			1907.	1907.	
Jan. 2	Mambusao	Capiz	Jan. 11	Jan. 26	26
Feb. 13	Saravia	Occidental Negros		Feb. 29	9
Feb. 26	Sigma	Capiz		Jan. 29	2
Mar. 12	Pilar	Occidental Negros		Mar. 16	9
Mar. 23	Bacolod	do		Mar. 23	2
Apr. 7	Victoria	do		Apr. 29	15
	Parañaque	Rizal			25
	Pililia	do			28
	Navotas	do			140
	Bacoor	Cavite			18
	Indang	do			21
	Calawang	La Laguna			1
	Lingayen	Pangasinan			1
	Total number cases				7,085

JULY TO DECEMBER, 1907.

July 22	Carigara	Leyte	Aug. 25	Sept. 8	86
Aug. 9	Mandaon	Masbate	Sept. 12	Oct. 4	53
Aug. 18	Bamgo	Leyte	Sept. 7	Sept. 13	10
Oct. 6	Baliuag	Bulacan	Nov. 15	Dec. 11	10
Nov. 3	Calumpit	do	Nov. 30	Dec. 7	16
Nov. 10	San Miguel	do	Dec. 6	Dec. 10	26
Nov. 14	Guagua	Pampanga	Dec. 14	Dec. 14	24
Nov. 14	Sexmoan	do	Dec. 8	Dec. 12	42
Nov. 19	Santa Rita	do	Nov. 19	Dec. 5	4
Nov. 26	México	do	Nov. 26	Nov. 26	1
Nov. 29	Apalit	do		Dec. 11	9
Nov.	Bacolor	do	Dec. 7	Dec. 13	46
Nov.	San Fernando	do	Dec. 12	Dec. 14	10
Nov.	Macabebe	do	Dec. 6	Dec. 12	52
Nov.	Betis	do		Dec. 8	4
Nov.	San Luis	do	Dec. 6	Dec. 6	2
Dec. 1	Orion	Bataan	Dec. 1	Dec. 4	2
Dec. 1	Lubao	Pampanga	Dec. 15	Dec. 31	27
Dec. 2	Paombong	Bulacan	Dec. 10	Dec. 11	13
Dec. 2	Quingua	do	Dec. 7	Dec. 10	3
Dec. 2	Bulacan	do	Dec. 4	Dec. 7	2
Dec. 2	Mabalacat	Pampanga	Dec. 2	Dec. 2	1
Dec. 3	Candaba	do	Dec. 3	Dec. 3	1
Dec. 4	Hagonoy	Bulacan	Dec. 4	Dec. 4	3
Dec. 4	Bocaue	do	Dec. 5	Dec. 10	5
Dec. 10	Balanga	Bataan	Dec. 10	Dec. 10	1
Dec. 23	Malolos	Bulacan	Dec. 10	Dec. 11	7
	Florida	Pampanga			3
	Minalin	do			8
	Caloocan	Rizal			6
	Malabon	do			1
	Total				478

TABLE II.—*Cases and deaths from cholera in the city of Manila, January 1, 1906, to January 1, 1907.*

BY AGES.

Age.	Cases.	Deaths.	Mortality.
Under 30 days	1	1	100
1 month to 2 years	39	36	92.3
2 to 5 years	121	116	95.9
5 to 10 years	54	50	92.6
10 to 15 years	56	43	76.8
15 to 20 years	97	75	77.3
20 to 25 years	112	96	85.7
25 to 30 years	113	100	97.4
30 to 35 years	71	57	80.3
35 to 40 years	61	55	90.2
40 to 45 years	24	22	91.3
45 to 50 years	35	32	91.4
50 to 55 years	18	17	94.4
55 to 60 years	22	22	100
60 to 65 years	5	5	100
65 to 70 years	11	11	100
70 to 75 years	2	1	50
75 to 80 years	3	3	100
80 to 85 years	1	0	0
85 to 90 years	2	2	100
Over 90 years			
Total	848	744	87.7

BY RACE.

Race.	Cases.	Deaths.	Mortality.	1 case to—	Date last case.
Americans	12	9	75	488	Sept. 4, 1906
Filipinos	782	698	89.3	243	Nov. 17, 1906
Chinese	30	27	90	741	Oct. 12, 1906
Foreigners	24	10	41.7	189	Nov. 27, 1906

TABLE III.—*Cases and deaths from cholera in the city of Manila, January 1, 1907, to January 1, 1908.*

BY AGES.

Age.	Cases.	Deaths.	Mortality.
Under 30 days			
1 month to 2 years	6	6	100
2 to 5 years	14	13	92.85
5 to 10 years	15	14	93.33
10 to 15 years	12	10	83.33
15 to 20 years	21	14	66.66
20 to 25 years	30	28	93.33
25 to 30 years	40	32	80
30 to 35 years	26	24	92.30
35 to 40 years	21	20	95.23
40 to 45 years	16	13	81.25
45 to 50 years	8	8	100
50 to 55 years	7	7	100
55 to 60 years	3	1	33.33
60 to 65 years	2	2	100
65 to 70 years	2	2	100
70 to 75 years			
75 to 80 years			
80 to 85 years			
85 to 90 years			
Over 90 years			
Total	223	194	86.99

BY RACE.

Race.	Cases.	Deaths.	Mortality.	1 case to—	Date last case.
Americans	3	0	0.0	1,733	Oct. 17, 1907
Filipinos	212	186	87.7	921	Dec. 31, 1907
Chinese	4	4	100	4,507	Oct. 19, 1907
Foreigners	4	4	100	1,255	Oct. 9, 1907
Total	223	194	86.9	1,002	

ILLUSTRATIONS.

PLATES I AND II. Houses in the Farola district where cholera first appeared in 1902.
MAP No. 1. Portion of Philippines including Manila Bay and Laguna de Bay.
2. The Philippine Islands, by provinces.

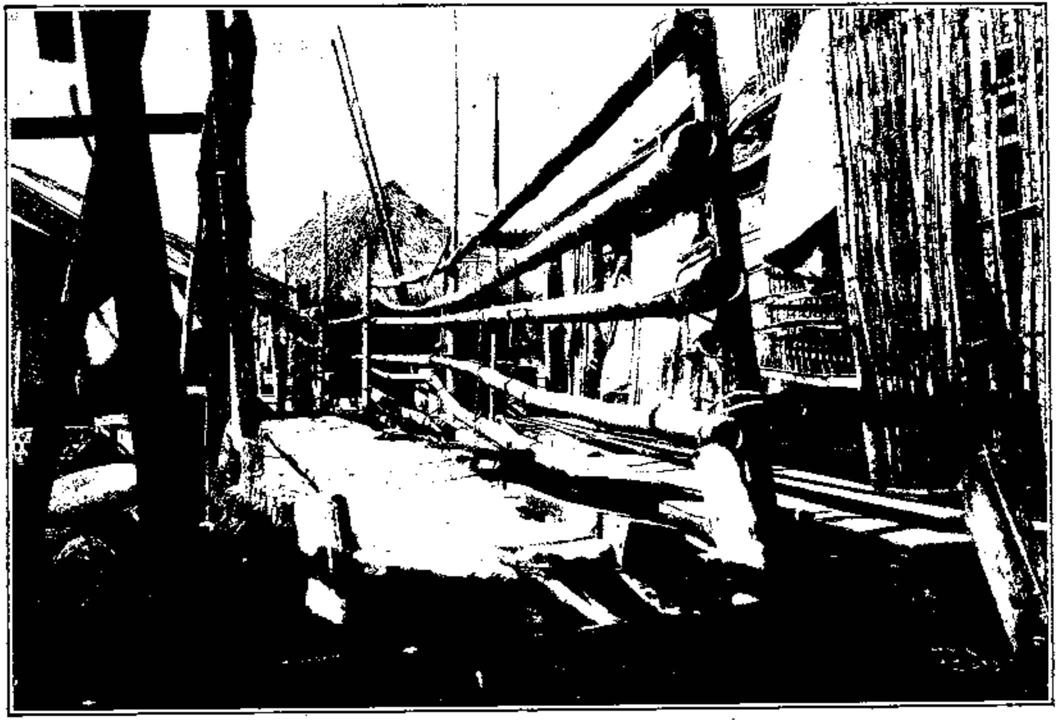


PLATE 1. HOUSES IN THE FAROLA DISTRICT WHERE CHOLERA FIRST APPEARED IN 1902.

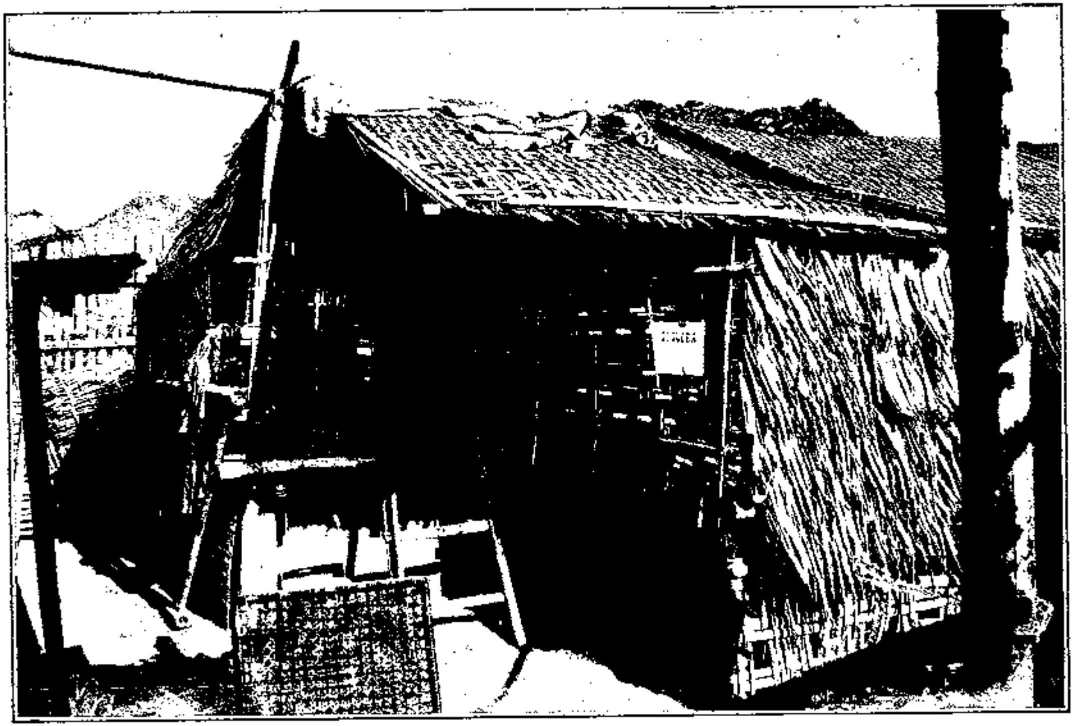
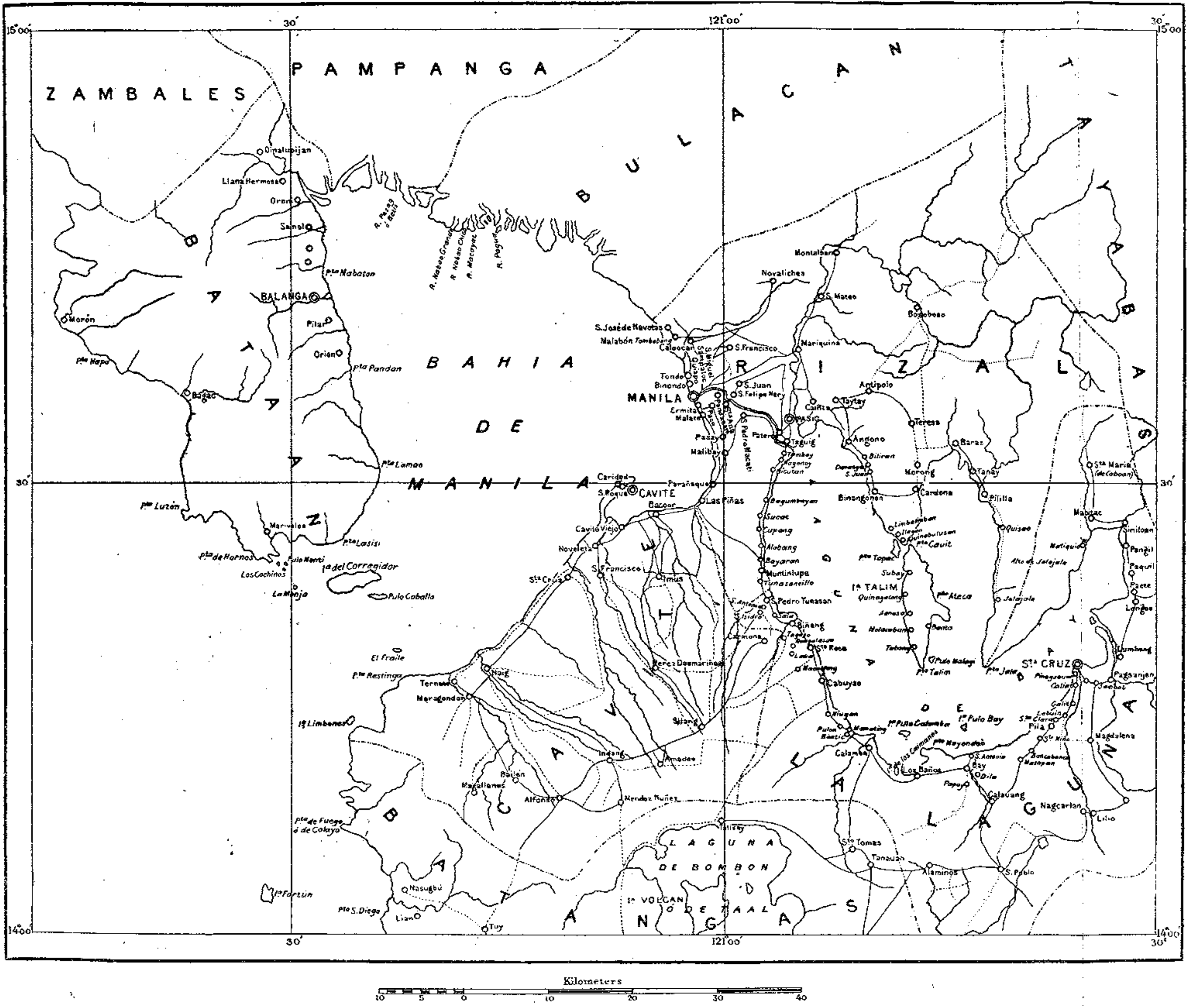
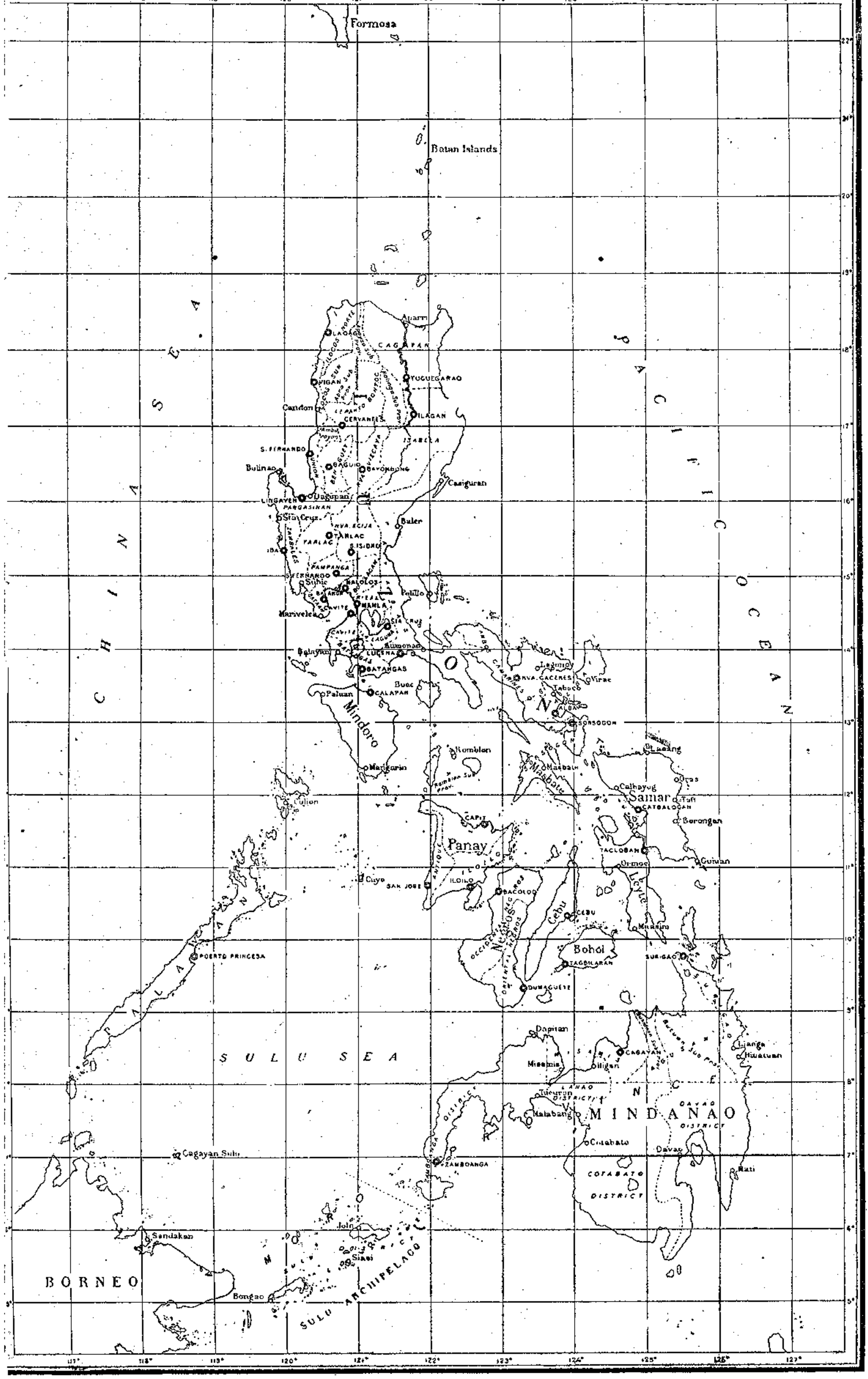


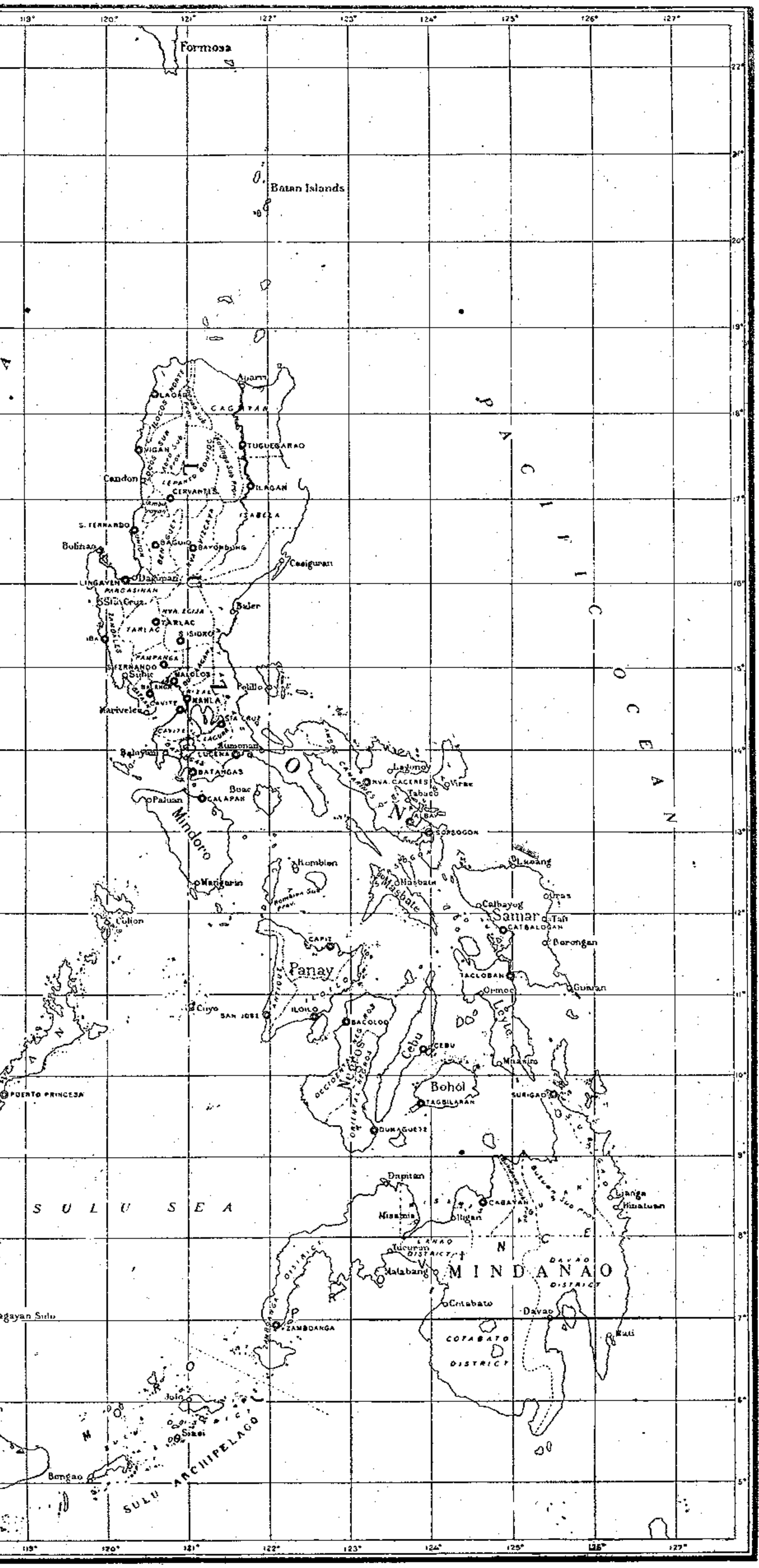
PLATE II. HOUSES IN THE FAROLA DISTRICT WHERE CHOLERA FIRST APPEARED IN 1902.


15°00'
30'
121°00'
30'
15°00'
ZAMBALES
PAMPANGA
BULACAN
TAYABAS
RIZAL
BATAAN
BAHIA DE MANILA
CAVITE
LAGUNA
LAGUNA DE BAY
BATANGAS
LAGUNA DE BOMBON Ó DE TAAL
Ia VOLCAN
Dinalupijan
Llana Hermosa
Orani
Samal
Pta Nabaton
BALANGA
Morón
Pilar
Orion
Pta Napo
Pta Pandan
Bagac
Pta Lamao
Pta Luzón
Mariveles
Pta de Hornos
Pulo Monti
Pta Lasisi
Los Cochinos
Ia del Corregidor
La Monja
Pulo Caballo
R. Pasag ó Betis
R. Nabao Grande
R. Nabao Chico
R. Macayat
R. Pagaa
Montalban
Novaliches
S. Mateo
Bosoboso
S. José de Navotas
Malabón
Tambobong
Caloocan
S. Francisco
Mariquina
Tondo
Binondo
S. Miguel
Sampaloc
Quiapo
S. Juan
S. Felipe Nery
MANILA
Ermita
Malate
Paco
Pandacan
Sta Ana
S. Pedro Macati
Pasay
Malibay
Pateros
Taguig
PASIG
Cainta
Taytay
Antipolo
Teresa
Angono
Baras
Bilibran
Tanay
Dorongan
S. Juan
Morong
Sta María (de Caboan)
Caridad
S. Roque
CAVITE
Parañaque
Las Piñas
Bacoor
Binangonan
Cardona
Pililla
Bagumbayan
Sucat
Cupang
Alabang
Bayanan
Muntinlupa
Tunasancillo
S. Pedro Tunasan
Limbabiuban
Ilagan
Quinabuluan
Pta Cavit
Pta Tapac
Subay
Ia TALIM
Quinagatan
Pta Ateca
Janosa
Malacaban
Tabong
Banto
Pulo Malaqui
Pta Talim
Pta Jala
Quisao
Mabitac
Siniloan
Matiquio
Alto de Jalajala
Jalajala
Panguil
Paquil
Paete
Longos
Lumbang
Cavite Viejo
Noveleta
S. Francisco
Imus
Sta Cruz
S. Antonio
S. Isidro
Biñang
Sala
Carmona
Tagala
Santa Rosa
Labac
Macabling
Cabuyao
Perez Dasmariñas
Naig
El Fraile
Pta Restinga
Ternate
Maragondon
Silang
Ia Limbones
Indang
Amadeo
Bailen
Magallanes
Alfonso
Mendez Nuñez
Pta de Fuego ó de Calayo
Ia Fortún
Nasugbú
Pta S. Diego
Lian
Tuy
Talisay
Sto Tomas
Tanauan
Alaminos
S. Pablo
Niugan
Pulan Bastic
Mamatiug
Ia Pulo Calamba
Ia Pulo Bay
Calamba
Pta Los Caimanes
Pta Mayondon
Los Baños
S. Antonio
Bay
Bilo
Papaya
Calauang
Nagcarlan
Liliw
Sta CRUZ
Pinagsauaan
Calios
Gatid
Labuin
Sta Clara
Pila
Sto Niño
Bañcabanca
Masapan
Pagsanjan
Santol
Magdalena
14°00'
30'
121°00'
30'
14°00'
Kilometers
10
5
0
10
20
30
40

MAP No. 1.



Formosa
Batan Islands
CHINA SEA
PACIFIC OCEAN
LUZON
Aparri
CAGAYAN
LAOAG
ILOCOS NORTE
ILOCOS SUR
VIGAN
Abra Sub Prov.
BONTOC
Apayao Sub Prov.
Candon
LEPANTO
CERVANTES
ILAGAN
YUGUEGARAO
Amburayan
ISABELA
S. FERNANDO
UNION
BAGUIO
BENGUET
BAYOMBONG
NVA. VIZCAYA
Bulinao
Casiguran
LINGAYEN
Dagupan
PANGASINAN
Sta. Cruz
NVA. ECIJA
TARLAC
Baler
ZAMBALES
IBA
S. ISIDRO
PAMPANGA
S. FERNANDO
Subic
MALOLOS
BULACAN
BALANGA
BATAAN
RIZAL
MANILA
Polillo
Mariveles
CAVITE
STA. CRUZ
LAGUNA
Atimonan
Balayan
BATANGAS
LUCENA
TAYABAS
AMBOS CAMARINES
Legaspi
NVA. CACERES
Virac
Tabaco
ALBAY
SORSOGON
Paluan
CALAPAN
Boac
Mindoro
Mangarin
Romblon
Romblon Sub Prov.
Masbate
Masbate
Culion
Catbalogan
Laoang
Calbayog
Samar
Oras
CATBALOGAN
Taft
Borongan
CAPIZ
Panay
ANTIQUE
ILOILO
Cuyo
SAN JOSE
ILOILO
BACOLOD
TACLOBAN
Ormoc
Guiuan
Leyte
Maasin
CEBU
Cebu
Negros
OCCIDENTAL NEGROS
ORIENTAL NEGROS
Bohol
TAGBILARAN
SURIGAO
DUMAGUETE
PALAWAN
PUERTO PRINCESA
SULU SEA
Dapitan
CAGAYAN
MISAMIS
Misamis
Iligan
Butuan Sub Prov.
AGUSAN
SURIGAO
Lianga
Hinatuan
LANAO DISTRICT
Tucuran
Malabang
MINDANAO
DAVAO DISTRICT
ZAMBOANGA DISTRICT
ZAMBOANGA
Cotabato
Davao
COTABATO DISTRICT
Mati
Cagayan Sulu
Jolo
Siasi
SULU ARCHIPELAGO
MORO
Sandakan
BORNEO
Bongao
5°
6°
7°
8°
9°
10°
11°
12°
13°
14°
15°
16°
17°
18°
19°
20°
21°
22°
117°
118°
119°
120°
121°
122°
123°
124°
125°
126°
127°


119°
120°
121°
122°
123°
124°
125°
126°
127°
22°
21°
20°
19°
18°
17°
16°
15°
14°
13°
12°
11°
10°
9°
8°
7°
6°
5°
Formosa
Batan Islands
PACIFIC OCEAN
Aparri
LAOAG
ILOCOS NORTE
CAGAYAN
VIGAN
ILOCOS SUR
TUGUEGARAO
Candon
LEPANTO
BONTOC
CERVANTES
ILAGAN
ISABELA
S. FERNANDO
UNION
BENGUET
BAGUIO
BAYOMBONG
NVA. VIZCAYA
Bolinao
Casiguran
LINGAYEN
Dagupan
PANGASINAN
Sta. Cruz
NVA. ECIJA
Baler
TARLAC
S. ISIDRO
IBA
ZAMBALES
PAMPANGA
S. FERNANDO
Subic
MALOLOS
BULACAN
BATAAN
RIZAL
Polillo
MANILA
Mariveles
CAVITE
LAGUNA
STA. CRUZ
Balayan
LUCENA
TAYABAS
Lumonan
BATANGAS
Boac
CALAPAN
Paluan
Mindoro
Mangarin
LUZON
AMBOS CAMARINES
Lagonoy
NVA. CACERES
Virac
Tabaco
ALBAY
SORSOGON
Romblon
Romblon Sub Prov.
Masbate
Laoang
Catbalogan
Calbayog
Samar
Oras
Taft
CATBALOGAN
Borongan
Culion
CAPIZ
Panay
ANTIQUE
ILOILO
TACLOBAN
Ormoc
Guiuan
Leyte
Cuyo
SAN JOSE
ILOILO
BACOLOD
Negros
OCCIDENTAL NEGROS
ORIENTAL NEGROS
Cebu
CEBU
Maasin
Bohol
TAGBILARAN
SURIGAO
PUERTO PRINCESA
DUMAGUETE
SULU SEA
Dapitan
Misamis
CABAYAN
Iligan
Liangan
Hinatuan
LANAO DISTRICT
Tucuran
Malabang
MINDANAO
DAVAO DISTRICT
Cotabato
Davao
COTABATO DISTRICT
Mati
ZAMBOANGA
Cagayan Sulu
Jolo
Siasi
Bongao
SULU ARCHIPELAGO
MORO PROVINCE

STUDIES OF CHOLERA.[1]

By HARRY T. MARSHALL.
(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

I. VARIATIONS IN VIRULENCE.

During the past year or more we have been continuing the studies of cholera at this laboratory, previous reports upon this subject having been published by Dr. Strong. In this paper I will review the results obtained up to the present time.

The experiments which I have conducted were undertaken with the view of continuing certain investigations upon the variation of bacteria with special reference to their so-called immunization. This research was begun a few years ago with the dysentery bacillus.[2] At that time we came to the conclusion that selection and variation are constantly manifesting their action upon bacteria, and that these processes probably play an important part, not only in the rise of epidemics, but also in the development of each individual case of an infectious disease, in such a manner that in a case of infection a large number of the bacteria are killed by the host, while a relatively small number become adapted to their environment, survive, multiply and produce the disease. At that time, we were able by growing the dysentery bacillus upon media containing anti-dysenteric serum, to develop a variety of bacillus which not only was not agglutinated or impeded in its growth by the antiserum, but which actually flourished more luxuriantly in the presence of the serum than it did under other conditions and which no longer united with the agglutinins in specific antidysenteric serum. A preliminary attempt was made in this laboratory to repeat the same experiment with the cholera vibrio. Two or three stock cultures were inoculated into bouillon or peptone solutions containing small amounts of anticholera serum and after intervals varying from a day up to a week or more, transplants were made into fresh flasks of bouillon containing anticholera serum. As many as ten successive transplants in one series were made after this fashion, but the cholera vibrio did not lose its capacity to become agglutinated, although it grew readily in the new medium. This line of investigation was not carried any further.

---

[1] Read at the Fifth Annual Meeting of the Philippine Islands Medical Association, February 28, 1908.

[2] Marshall and Knox: *J. Med. Research* (1906), N. S. 10, 325.

One interesting observation was made upon a strain which had been transplanted nine times through successive serum bouillon cultures and which had been allowed to remain in the last flask for a long period. The vibrio was plated out and its biological characteristics determined. Among other distinctive features the blood-agar-hæmolysis test was tried simultaneously with this bacterium and the stock culture from which it was taken for the original transplant into serum. The original stock culture showed no hæmolysis within twenty-four hours and only a trace in forty-eight hours, while the vibrio which had been grown in beef broth, containing anticholera horse serum, showed a marked ability to produce hæmolysis of goat's blood within twenty-four hours. We have here a definite variant produced by artificial laboratory means, the only difference between the stock culture and the variant being that the variant grew in the presence of serum from a horse which had been immunized against cholera. The factor which produced the hæmolyzing reaction was probably not the specific anticholera substance of the serum but those constituents of it which are close to the ones of the red blood corpuscles.

The question of variation was then attacked in a different way by Dr. Philip K. Gilman, of the Philippine Medical School, and myself. We created an artificial epidemic of cholera in guinea pigs in order to observe what changes the vibrio would undergo both in virulence and in other respects. A guinea pig received an inoculation intraperitoneally of 0.1 cubic centimeter of the material taken from the ileum of a man dead of cholera, the diagnosis being based upon clinical history, autopsy appearances and laboratory examinations. The guinea pig died within twenty-four hours; 0.1 cubic centimeter of the peritoneal contents from this animal, diluted with salt solution, was inoculated into the peritoneal cavity of a second guinea pig, and upon its death, within twenty-four hours, the peritoneal exudate was used for inoculating a third. Upon its death within twenty-four hours, the exudate was diluted and a series of guinea pigs was inoculated with varying amounts of this material. The minimum dose fatal within twenty-four hours was 0.02 cubic centimeter. The exudate from the guinea pig killed by this amount was diluted and a fresh series inoculated. On this occasion, although as small a dose as 0.0005 cubic centimeter was inoculated, the minimal fatal dose was not determined. The exudate from the animal killed by the lowest fatal dose was used for inoculating a sixth series and in this 0.0001 cubic centimeter was the minimal fatal dose. From the exudate obtained from the animal which succumbed to this quantity a new series was inoculated and 0.00002 cubic centimeter proved fatal. An interesting result followed the next inoculation. The exudate was diluted and inoculated as usual, but none of the guinea pigs died within twenty-four hours, although the doses inoculated varied from 0.001 to 0.00001 cubic centimeter. In other words, the virulence had suddenly fallen at least to one-fiftieth of its former strength, and how much more is not known. It is important to determine the nature of the biological processes associated with this sudden abatement of virulence, but the only clue furnished by this series is that 1 cubic centimeter of a 1 to 1,000,000 dilution of the exudate from the next to the last guinea pig of the series, when plated in agar, contained colonies too numerous to count, so that at least it is clear that the bacteria had not lost their viability. We may account for the sudden loss of virulence either by supposing that the power of toxin formation suddenly disappeared, or that the ability of the bacteria to resist bactericidal action suddenly failed them. It seems clear that there was a sufficient number of surviving bacteria carried over from the next to last guinea pig; this was shown by the results of plating. The results of this series of inoculations are shown in Series I, and the sudden rise and fall in virulence is charted in fig. 1.

The strain of cholera which has just been described was obtained from an autopsy during the height of a small epidemic. Several other attempts were made to repeat this result subsequently, when the epidemic was on the wane, both with strains of cholera inoculated into guinea pigs directly from the intestine and with laboratory stock cultures. The results are shown in Series II, III, IV, V, and VI and in figs. 2–5. It is seen that no strain was obtained, the virulence of which could be increased so successfully and regularly as that of the first.

Turning to Series II, we find the results are less regular, although here again there was a gradual rise in virulence up to a fatal dose of 0.0001 cubic centimeter. The irregularities are difficult to explain. For instance, we find on November 11 that 0.0002 cubic centimeter is fatal, while 0.001 is not so. Again, on November 13 neither 0.01 nor 0.0002 cubic centimeter killed the animal, while 0.0001 and 0.001 did so. It is possible that this difference can be explained upon a physical basis, for the exudate was tenacious and possibly did not spread evenly throughout the diluting salt solution.

The virulence was irregular in Series III also. An observation made in this series throws some light upon the mechanism of the fall of an epidemic. In the fifteenth inoculation made on November 28, the only animal which died within twenty-four hours received 0.01 cubic centimeter of exudate. This animal did not die until 10.15 on the morning of November 29. The autopsy was performed at once and 0.25 cubic centimeter of tenacious, yellowish, cloudy exudate was recovered from the peritoneal cavity. As the table shows, the animals inoculated with this exudate did not die within twenty-four hours. At the same time 1 cubic centimeter of a 1 to 80 dilution of this exudate was inoculated into another guinea pig and at intervals a few drops of peritoneal fluid were drawn off and plated. One one-thousandth cubic centimeter of the fluid drawn after half an hour contained two colonies, and the fluid drawn after three and one-half and after twenty-four hours was sterile, while a control plate made with one four hundred thousandth of the exudate used for inoculation showed thirty-eight colonies, and another control with one four thousandth cubic centimeter developed about two hundred and seventy-six colonies.

This must be interpreted as proving that the bacteria in the exudate were no longer viable in the guinea pig's peritoneal cavity, although still viable on artificial media.

Reference to the table will show that the cultures and morphology of the peritoneal bacteria were not typical, although cholera-like organisms were found. However, the fact that in the inoculations of November 27 the minimal fatal dose was 0.0001 cubic centimeter and that the exudate from this animal gave a surface growth on peptone, indicate that the cholera vibrios were still living up to the next to last of the series, and it is a reasonable presumption that they lived up to the last inoculation. This finding contrasts rather strikingly with the condition observed at the close of Series I.

Series IV, inoculated from an autopsy toward the close of the epidemic, showed such low pathogenic power that 0.1 cubic centimeter of a 24-hour peptone culture did not kill a guinea pig. After Series V had been continued four days, the virulence fell on the fifth below 0.01, and the series was discontinued. With Series VI also the virulence rose to 0.01, then fell.

These experiments show that there is a great difference between the individual strains of cholera, and that this difference is a deep-seated one, possibly fundamental. They also demonstrate the possibilities of variation with cholera to be enormous; for here we have the fatal dose for medium-sized guinea pigs one fifty-thousandth and one ten-thousandth of a cubic centimeter of exudate, which minute quantity is not made up entirely of bacteria but contains also serum and cellular contents, while the average standard for cholera as given by Kolle is one-tenth of a platinum loopful, which would be approximately 2 milligrams.

DIFFERENCES DURING ROUTINE EXAMINATIONS.

It may be well to refer briefly at this point to certain differences which have been noted during the routine examinations upon cases suspected of cholera. In the epidemic of August, 1907, routine examinations were made, according to custom, of the bowel contents of all cases supposed to have died of cholera and of the stools of patients supposed to have been suffering from this disease. At that time Dr. Ralph T. Edwards, of this laboratory and I, noted that during the height of the epidemic the vibrios presented the typical morphology, were actively motile and agglutinated in a high dilution of serum; while toward its close the curves of the vibrios became less evident, many forms were found with little or no curve, and the bacteria in general were slenderer and longer; the motility diminished greatly and the movement, if present at all, was very sluggish, while the agglutinatibility remained practically constant. In two patients who recovered from cholera, examined at the close of this epidemic, the vibrios remained in the stools after convalescence; in one case for four or five days, while in the other they were still present after ten days, when the patient left the hospital and was lost sight of. The importance of this occurrence in its bearing upon the spread of cholera is sufficiently evident to make comment superfluous. This case recalls at once the findings of Ruffer and Gotschlich, which will be discussed in a later section of this report.

The study of the bacterial variation occurring among cholera vibrios is being pursued along other lines also. A number of different strains of cholera and cholera-like vibrios have been collected and a comparison is being made of their pathogenicity, motility, morphological and cultural characteristics, serum reactions, and ability to form toxins. The results of these studies will be reported later. Up to the present time the chief attention has been devoted to noting the cultural characteristics, and there is evidence of appreciable differences in the manner of growth of the various organisms which are being examined.

SERIES I.—*From cholera autopsy, September 4, 1907, contents of ileum direct into guinea pig intraperitoneally.*

[+ = fatal in 24 hours after inoculation. 0 — not fatal in 24 hours after inoculation.]

Date of inoculation.	Volume (in fractions of a cubic centimeter) of exudate from dead guinea pigs which proves fatal in less than 24 hours after intraperitoneal inoculation into a new set of guinea pigs.														Appearance of exudate.	Cultures, smears, etc., from exudate.	Remarks.
	0.2.	0.1.	0.05.	0.02.	0.01.	0.005.	0.002.	0.001.	0.0005.	0.0002.	0.0001.	0.00005.	0.00002.	0.00001.			
Sept. 4		+[a]															
Sept. 5		+															
Sept. 6		+															
Sept. 7	+	+	+	+	0	0	0	0	0								
Sept. 8				+	+	+	+	+	+								0.0005 cc. fatal in less than 24 hours; minimum fatal dose not reached.
Sept. 9									+	+	+	0	0	0	4 cc. of thick tenacious exudate.	1 cc. of 1 to 100,000 dilution plated in agar; colonies too numerous to count.	
Sept. 10								+	([b])	([b])	+	+	+	0	0.00002 has 1.5 cc. thick tenacious exudate.	1 cc. of 1 to 1,000,000 dilution plated in agar; colonies too numerous to count.	
Sept. 11								0	([b])	([b])	([b])	0	0	0			

[a] A second guinea pig receiving 0.1 cubic centimeter of bowel contents did not die until the third day.

[b] Not inoculated.

September 7, 1907, was the first day upon which a series of several guinea pigs was inoculated.

SERIES II.—*Inoculations started from a 24-hour bouillon culture of a stock laboratory culture of cholera.*

[+ = fatal within 24 hours after inoculation. 0 = not fatal within 24 hours after inoculation.]

Date of inoculation.	Volume (in fractions of a cubic centimeter) of exudate from dead guinea pigs which proves fatal in less than 24 hours after intraperitoneal inoculation into a new set of guinea pigs.														Appearance of exudate and peritoneal surfaces.	Cultures, smears, etc., from exudate.	Remarks.
	0.5.	0.1.	0.05.	0.02.	0.01.	0.005.	0.002.	0.001.	0.0005.	0.0002.	0.0001.	0.00005.	0.00002.	0.00001.			
Oct. 30	+	0	0	0	0	----	----	----	----	----	----	----	----	----	12 cc. turbid yellow mucoid exudate.	----	Fatal dose of *stock cholera culture.*
Nov. 6	----	----	----	----	----	----	----	----	----	----	----	----	----	----	Hæmorrhagic exudate.	----	Received 2 cc. of peptone culture from exudate Oct. 30.
Nov. 7	----	+	----	----	----	----	----	----	----	----	----	----	----	----	Injected peritoneum; fibrin flakes; 1 cc. turbid bloody exudate.	----	
Nov. 8	----	+	+	+	0	0	+	0	0	----	----	----	----	----	0.002 guinea pig contains 1¼ cc. bloody peritoneal exudate.	----	Guinea pig dying after a dose of 0.002 was pregnant.
Nov. 9	----	----	----	+	----	0	0	0	+	+	----	----	----	----	Petechiæ over colon; a few fibrin flakes over peritoneum; turbid red exudate.	----	
Nov. 10	----	----	----	----	+	+	----	0	----	0	0	0	0	----	----	----	
Nov. 11	----	----	----	----	+	----	----	0	----	+	0	0	0	----	----	----	
Nov. 12	----	----	----	----	+	----	----	+	0	0	0	0	----	----	Slight rosy coloration; peritoneal cavity almost dry: no fibrin: no extravasation.	Plates and slants show pure culture slightly curved rods.	
Nov. 13	----	----	----	----	0	----	----	+	----	0	+	0	0	----	3½ cc. bright red tenacious fluid.	Peptone gives general turbidity and sediment.	Plates, and slants apparently pure culture of slightly curved rods.
Nov. 14	----	----	----	----	+	----	----	0	----	+	0	0	0	----	5 cc. turbid bloody fluid.	No surface growth on peptone.	No growth in alkaline agar plates. Series discontinued.

SERIES III.—*Inoculation made from the bowel contents of a fatal case of cholera directly into peritoneal cavity of guinea pig.*

[+ = fatal within 24 hours after inoculation. 0 = not fatal in 24 hours after inoculation.]

Date of inoculation.	Volume (in fractions of a cubic centimeter) of exudate which proved fatal within 24 hours after intraperitoneal inoculation into guinea pigs.														Appearance of exudate and peritoneal surfaces.	Cultures, smears, etc., from exudate.	Remarks.
	0.2.	0.1.	0.05.	0.02.	0.01.	0.005.	0.002.	0.001.	0.0005.	0.0002.	0.0001.	0.00005.	0.00002.	0.00001.			
Nov. 14	----	----	----	----	----	----	----	----	----	----	----	----	----	----	5 cc. turbid yellow exudate, large flakes of fibrin over peritoneal surfaces.	----	1 cc. of bowel content from fatal case cholera inoculated intraperitoneally.
Nov. 15	----	+	----	----	+	----	----	0	----	----	0	----	0	----	0.2 cc. reddish fluid. Injected peritoneum; fibrin flakes.	----	
Nov. 16	----	+	+	----	----	----	----	----	----	----	----	----	----	----	----	----	Not enough exudate for further dilutions.
Nov. 17	----	----	----	----	+	----	----	+	----	+	0	0	0	----	4 cc. turbid yellow viscous fluid fibrin.	Plates; apparently pure culture cholera.	
Nov. 18	----	----	----	----	+	----	----	0	----	0	0	0	0	----	8 cc. blood-stained fluid	Peptone; no pellicle; plates, slants, morphology = pure cholera vibrios with definite comma curve in over 50 per cent.	
Nov. 19	----	----	----	----	+	----	----	0	----	----	0	----	----	----	4.5 cc. bloody turbid exudate	Plates; apparently pure culture cholera.	
Nov. 20	----	----	----	----	+	----	----	+	----	----	0	----	----	----	3½ cc. bloody exudate; fibrin	Peptone and plates apparently pure culture cholera.	
Nov. 21	----	----	----	----	+	----	----	0	----	----	0	----	----	----	4 cc. bloody exudate	Plates and slants, pure culture of slender rods about 3 μ with irregular stain; 1 in 10 with curved tip.	
Nov. 22	----	----	----	----	+	----	----	+	----	----	0	----	----	----	2 cc. turbid red fluid	----	
Nov. 23	----	----	----	----	+	----	----	0	----	0	----	----	----	----	----	Peptone not typical	
Nov. 24	----	----	----	----	+	----	----	0	----	0	----	----	----	----	----	do	

Nov. 25	----	----	----	----	+	----	----	+	----	----	+	----	----	----	3¼ cc. viscous blood-tinged exudate.	Plates and morphology apparently pure culture cholera; 1 in 8 to 1 in 15 curved.	Limit of virulence not reached.
Nov. 26	----	----	----	----	+	----	----	+	----	----	0	0	0	----	3 to 4 cc. bloody turbid fluid	Peptone not typical, i. e., no pellicle.	
Nov. 27	----	----	----	----	+	----	----	0	----	----	+	0	0	0	¼ cc. bloody turbid fluid	Peptone gives surface growth	
Nov. 28	----	----	----	----	+	----	----	0	----	0	0	0	----	----	¼ cc. yellow very tenacious exudate.	Rods 1 to 3 μ in length; irregular staining, about 1 in 20 show slight curve.	
Nov. 29	----	----	----	----	0	----	----	0	----	----	0	0	0	----	----	----	

SERIES IV.—*Inoculated from surface growth of peptone culture—24 hours old—from ileum of cholera autopsy.*

[+ = fatal within 24 hours after inoculation. 0 = not fatal in 24 hours after inoculation.]

Date.	Dilutions (in fractions of a cubic centimeter) of peptone culture used for intraperitoneal inoculation of guinea pigs.														Remarks.
	0.2.	0.1.	0.05.	0.02.	0.01.	0.005.	0.002.	0.001.	0.0005.	0.0002.	0.0001.	0.00005.	0.00002.	0.00001.	
Nov. 23	----	0	----	----	0	----	----	0	----	----	----	----	----	----	All animals survived. Series discontinued.

Series V.—*Inoculated intraperitoneally with red gruel-like bowel contents from cholera autopsy.*

[+ = fatal within 24 hours after inoculation. 0 = not fatal in 24 hours after inoculation.]

Date.		Volume (in fractions of a cubic centimeter) of exudate from dead guinea pig which proved fatal within 24 hours after intraperitoneal inoculation into guinea pigs.														Exudate, etc.	Cultures, smears, etc.	Remarks.
		1 cc.	0.1.	0.05.	0.02.	0.01.	0.005.	0.002.	0.001.	0.0005.	0.0002.	0.0001.	0.00005.	0.00002.	0.00001.			
Dec.	5	+	+			0										¾ cc. viscous fluid; blood tinged	Mixed culture; a few motile curved rods.	Inoculated with contents of ileum.
Dec.	6		+			+			0		0	0	0			3 cc. yellow viscous substance	A few curved slowly motile rods in exudate.	
Dec.	7		+			0			0			0	0	0		Semipurulent; not viscous	Mixed culture; no vibrios found.	
Dec.	8		0			+			0			0				1½ cc. slightly tenacious purulent fluid.	1 or 2 curved rods amid about 200 bacteria of various shapes.	
Dec.	9					0			0			0						Series discontinued.

Series VI.—*Inoculation from bowel contents from cholera autopsy.*

Date.		Volume of exudate from dead guinea pig which proved fatal within 24 hours after intraperitoneal inoculation into guinea pigs.														Exudate, etc.	Cultures, smears, etc.	Remarks.
		1 cc.	0.1.	0.05.	0.02.	0.01.	0.005.	0.002.	0.001.	0.0005.	0.0002.	0.0001.	0.00005.	0.00002.	0.00001.			
Dec.	6	+	0			0										3 cc. brownish yellow fluid	Mixed culture; no vibrios seen	1 cc. of contents of ileum inoculated.
Dec.	7	+	+			0			0							1 cc. yellowish slightly tenacious fluid.	Mixed culture with very few sluggish curved rods.	
Dec.	8		+			+										¼ cc. yellow fluid		Limit of virulence not reached.
Dec.	9		0			0			0			0						Series discontinued.

ILLUSTRATIONS.

FIG. 1. Chart of the changes in virulence of cholera strain No. 1. (To accompany Series I.)
2. Chart of the changes in virulence of cholera strain No. 2. (To accompany Series II.)
3. Chart of the changes in virulence of cholera strain No. 3. (To accompany Series III.)
4. Chart of the changes in virulence of cholera strain No. 4. (To accompany Series IV.)
5. Chart of the changes in virulence of cholera strain No. 5. (To accompany Series V.)

Figure I.

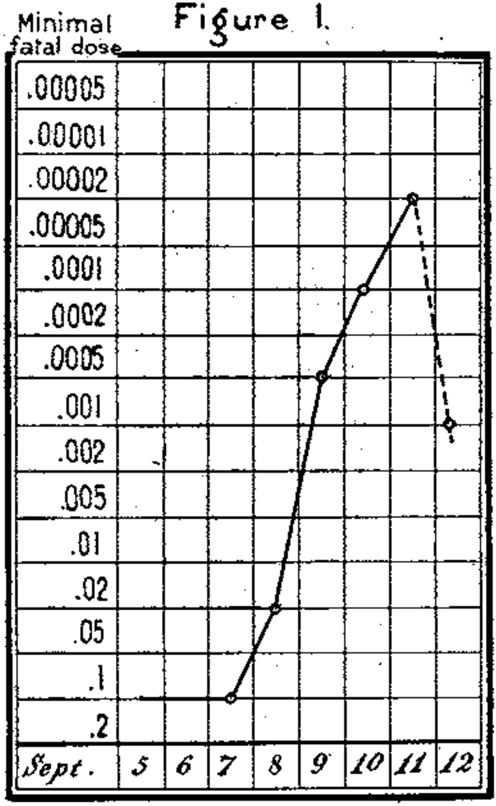


CHART OF THE CHANGES IN VIRULENCE OF CHOLERA STRAIN No. 1. (To accompany Series I.)

Figure II.

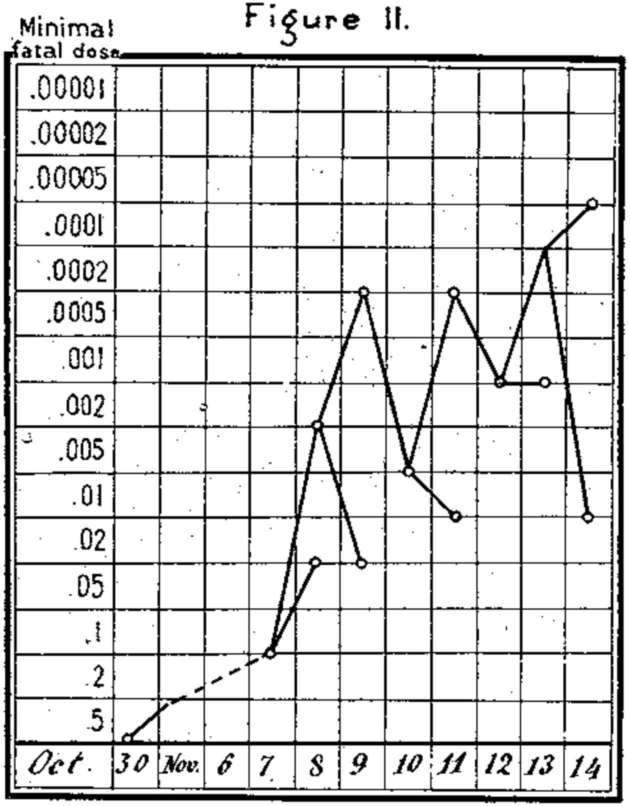


CHART OF THE CHANGES IN VIRULENCE OF CHOLERA STRAIN No. 2. (To accompany Series II.)

Figure III.

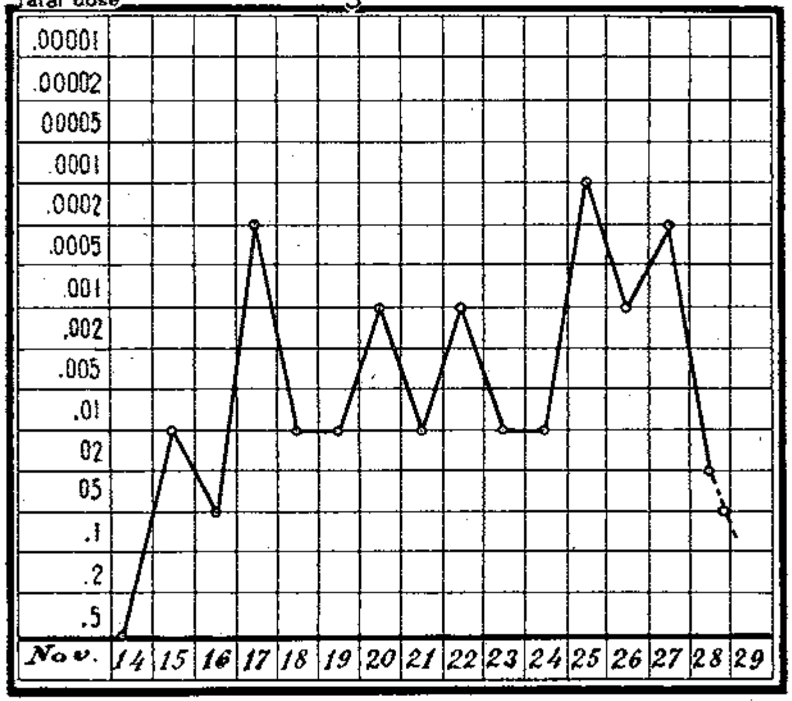


Chart of the changes in virulence of cholera strain No. 3. (To accompany Series III.)

Figure IV.

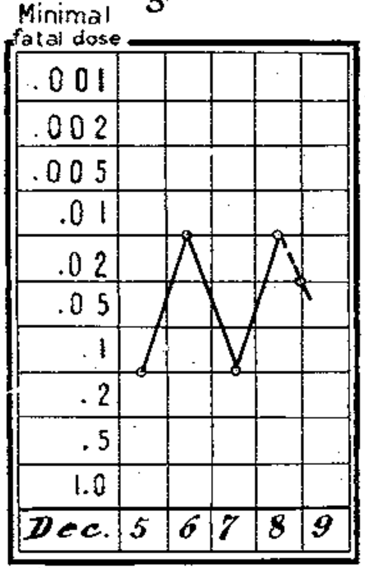


Chart of the changes in virulence of cholera strain No. 4. (To accompany Series IV.)

Figure V.

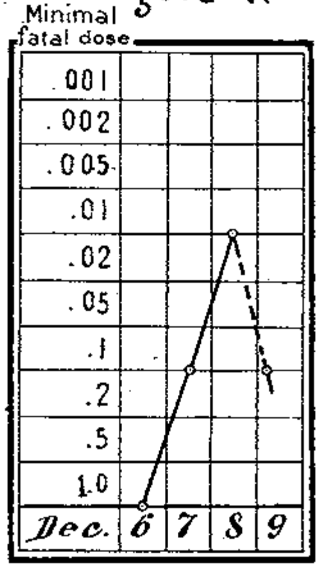


Chart of the Changes in virulence of cholera strain No. 5. (To accompany Series V.)

A BIOLOGICAL STUDY OF THE WATER SUPPLY OF THE PHILIPPINE ISLANDS, WITH A DESCRIPTION OF A NEW PATHOGENIC ORGANISM.[1]

By Ralph T. Edwards.

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

Manila at present is preparing to obtain an ample supply of drinking water and at the same time the city is very commendably applying itself to the task of making that supply safe. What will eventually be accomplished in this undertaking can be understood best by a study of the waters of the Islands, and therefore the statistics found in the official reports of water examinations made by the Bureau of Science up to January 1, 1908, are of great importance. These reports include waters from various places in the Islands and from divers sources in and about Manila; they include some 700 examinations made by Doctors Wherry, Woolley, Herzog, Bowman and Edwards, and by Messrs. Clegg and Willyoung.

This paper concerns itself first with the examination of waters from the *esteros*[2] because of the fact that while these are probably never drunk by the people here, laundry work is done on the estero banks and it is probable that dishes and cooking utensils are occasionally washed in those estuaries the odor of which is not too offensive. Examinations of flavoring extracts, soda water and bottled lemonade are also included for obvious reasons.

I have also taken up the subject of river water because our present supply for the city of Manila is derived from the Mariquina River, which flows into the Pasig some 6 miles above the mouth of the latter, and various other sources for drinking water are also included because of the reputation for purity which they enjoy in various places other than this city.

---

[1] Read at the Fifth Annual Meeting of the Philippine Islands Medical Association, February 29, 1908.

[2] Esteros are arms of the deltas of the Philippine rivers which are subject to tidal fluctuations and which are filled with salt water at high tide and are brackish at ebb tide. A number of these estuaries ramify through the city of Manila, the houses of the people are close to the banks and traffic crosses them by bridges. They are used by lighters, boats and other small craft. The current is sluggish.

Fifty-three per cent of all the 700 waters have been condemned for one cause or another. (See Chart I.) Of the total number examined, amœbæ were present in 39.4 per cent, but there were 46 per cent positive in those reports in which it was specifically stated that these organisms were searched for;[3] flagellata were encountered in 20 per cent of all waters, and the percentage of positive results on the reports in which these organisms are entered is 23.5; ciliata were mentioned as being present in 7.5 per cent and of the number in which protozoa were stated as being present or not, in 9.5 per cent; protozoa, without specification of identity, were mentioned in 1 per cent of all the waters and in 1.16 per cent of the number of completed examinations in which mention is made of such animal forms. High bacterial count was given as a reason for condemning a water in 9.3 per cent. However in 43 per cent of those cases where the fact of making such a count was mentioned, pathogenic bacteria were present in 0.83 per cent of the total, and in 28 per cent of all the cases in which a specific examination for these organisms was mentioned. Numerous special examinations for the presence of the cholera vibrio were undertaken, but up to January 1, 1908, no such specific organism was found, excepting in one estero where a railroad company had been emptying its sewage pails.

Chart II shows the percentage of condemned waters from various sources. The waters from public places included those from the courthouse, the public offices, the prison, restaurants and eating houses, excepting in instances where it was specifically stated that the sample examined was distilled water direct from one of the distilling plants. It is probable that if the number of samples of the latter class were to be subtracted from the total, the percentage of contaminated water would be much higher; as it is, it reaches 55 per cent. Fifty per cent of the spring water was condemned. The Manila supply, including samples from the intake, furnished the highest percentage—namely, 95.7. The study of the examinations from the latter source furnishes one of the strong arguments in favor of sand filtration. The colony count of the water secured from Manila hydrants varied from 40 to 600 bacteria in 1 cubic centimeter water. The low figure might be kept constant with an efficient sand filtration plant.

The samples of water passed through various household filters of divers construction gave 95 per cent to be condemned, the second highest number, but it is well to add that in nearly every instance representatives of this class were reported upon unfavorably because of the presence of animal forms. This result demonstrates the hopelessness of the expectation that a supply secured by filtration should be free from animal forms, but we know that water which has been subjected to slow sand filtration shows a very low number of bacteria and therefore such a process would greatly lessen the danger of *bacterial* infection for those who refuse to sterilize the hydrant water by some means before using it. Some experiments in sand filtration have been carried on in Manila, but these were only in mechanical filtration and left out of account the biological principle upon which all sand filtration should be based. The experiments of Musgrave and Clegg show that amœbæ require some organism to be in symbiosis with them, hence it is not difficult to believe that a reduction in the number of amœbæ would follow a diminution in the number of symbiotic organisms which, in this case, would be the bacteria in the water. It is a disputed point whether or not all amœbæ are pathogenic. If they are not, it would follow that by means of the reduction of the number of bacteria we might eliminate the pathogenic ones and so by the diminution of the total number of amœbæ, escape those which are pathogenic. Aside from this factor we know that natural immunity, or the sterilizing action of a healthy stomach, is able to a certain extent to protect individuals from intestinal bacterial infections. We know that many people in these Islands consistently refuse to sterilize the drinking water which they use, and a much larger number have their dishes washed only in unsterilized tap water.

---

[3] That is, reports in which amœbæ are especially mentioned as "positive" or "negative."

Only a few years ago, the prevalence of amœbic dysentery among Europeans was so great as seriously to reflect upon the Islands as a home for the Caucasians. However, the experience then gained was followed by the adoption of great precautions by this part of the population so far as purity of food and water was concerned, but now many persons who have had this experience have left, or have to a slight degree become immune to the ordinary chances of infection and the remainder are correspondingly careless. New arrivals are constantly appearing, and with such changes in the population and with certain other factors which we do not understand, we may probably have another epidemic at some future time, but we can furnish an improvement in the future water supply in the shape of a slow sand filtration plant. This would also probably help in the continual fight against cholera in Manila.

Few samples of waters bottled and for sale have been condemned and these, with one exception, were cheap products sold from low-class shops; only one sample of a reputable brand of water was condemned for high bacterial count, while no other of the same brand of water had even ten bacteria to the cubic centimeter, hence we would conclude that reputable brands of bottled water are safe.

No flavoring extract was condemned and only a few samples of soda water and lemonade, but the number of specimens examined was so small that we should draw no conclusions therefrom.

The specimens from artesian wells gave most satisfactory results, as only 8.5 per cent of the wells were condemned. The high figure 15.8

represents the proportion of samples at first rejected, but in some cases later investigation made with great precautions forced the conclusion that the samples were condemned in the first place because of contamination in securing the specimen.

Wells and cisterns showed 63.3 per cent of rejections and I do not doubt but that this number is far too low, for in instances the request for examination did not specify whether the sample was from an ordinary or an artesian well.

The miscellaneous places mentioned in the table include rivers, esteros, rice paddies, ponds, pools, ditches and tanks. Many of the requests for examination of samples called for only a partial examination for the presence of the cholera organism, and this accounts for the low percentage condemned.

Water should be condemned (*a*) for the presence of animal parasites, (*b*) for the presence of pathogenic bacteria, (*c*) when the count is above 200 bacteria per cubic centimeter.

The specimens of distilled water condemned were almost without exception rejected because of the presence of protozoa. It would, therefore, be legitimate to conclude that, in the handling of water, there is almost as much danger of contamination with protozoa as there is with bacteria and possibly this contamination is accompanied by greater danger to those who use the water for drinking purposes than the other. It is therefore advisable, in order to insure safety of drinking water, to make as few transfers from one receptacle to another as is possible after sterilization. I have already mentioned the good condition of reliable brands of water bottled and for sale, but can not condemn too strongly the handling which is ordinarily given to distilled and other potable waters when secured in large quantities.

A study of the above reports causes me to advocate a triple biological examination of all samples of water as follows:

> Twenty-five cubic centimeters of a 10 per cent peptone solution are added to about 225 cubic centimeters of water in a sterile flask (about the amount probably drunk by an individual at one time). The growth should be examined after twenty-four, forty-eight and seventy-two hours, with due precaution against contamination upon opening for the first two examinations. The report should mention amœbæ, flagellata and ciliata.
>
> Two cubic centimeters should be withdrawn with a sterile syringe at the 24-hour examination and injected into the peritoneal cavity of a guinea pig or rabbit; if the animal does not die within forty-eight hours it is quite probable that there are no pathogenic bacteria present. Plates should be made from the original peptone culture, containing 1, 0.1, 0.01 and 0.001 cubic centimeter of suspected water, respectively, and placed in the incubator for forty-eight hours before the colony count is made.

The figures appearing in this paper bring me to the conclusion that tap water, cistern water, ordinary well water, and water only filtered as a means of sterilization should not be used for drinking purposes in the

Philippines, nor in fact any other water excepting that which is known to be safe and is kept in closed sterile receptacles.

It is of interest to note that during the work on waters there was isolated from a specimen of water taken from a well on Calle Cabildo, Intramuros, a pathogenic bacillus of which I have been able to find no description. Its characteristics are as follows:

Bacillus cabildonis, spec. nov., 1908.

It is a single bacillus with a breadth of less than 1 μ, average length about 2 μ. It stains easily with the standard aqueous dyes and takes a polar stain with Z. N. carbol-fuchsin. It is decolorized by Gram's method. No capsule, spores, vacuoles, or crystals have been observed. The organism is actively motile and a facultative anaërobe. Pleomorphism has not as yet been observed. It grows well either at room or body temperature. A greenish fluorescence develops in twenty-four hours on all transparent media. The bacterium grows well on all ordinary media; nutrient broth remains alkaline as far as my observation goes, without the development of odor; milk is not coagulated, digested or acidified nor is an odor developed; no gas is formed with saccharose, glucose, glycerine or lactose; solidified blood serum is not peptonized; the growth on potato is abundant; indol is produced in sugar-free peptone solution; alkali is formed with or without the presence of carbohydrates. Guinea pigs or rabbits inoculated intraperitoneally with one or two drops of a 24-hour broth culture or with the peritoneal exudate of a previously inoculated animal, die in five or six hours with evidences of peritonitis and septicæmia. While this organism has been encountered but once, its extreme pathogenicity for small animals to my mind makes it of sufficient importance to merit description.

SUMMARY.

The presence of protozoa, pathogenic bacteria or a colony count of more than 200 per cubic centimeter being the criterion, 53 per cent of the waters heretofore examined in this laboratory should be condemned.

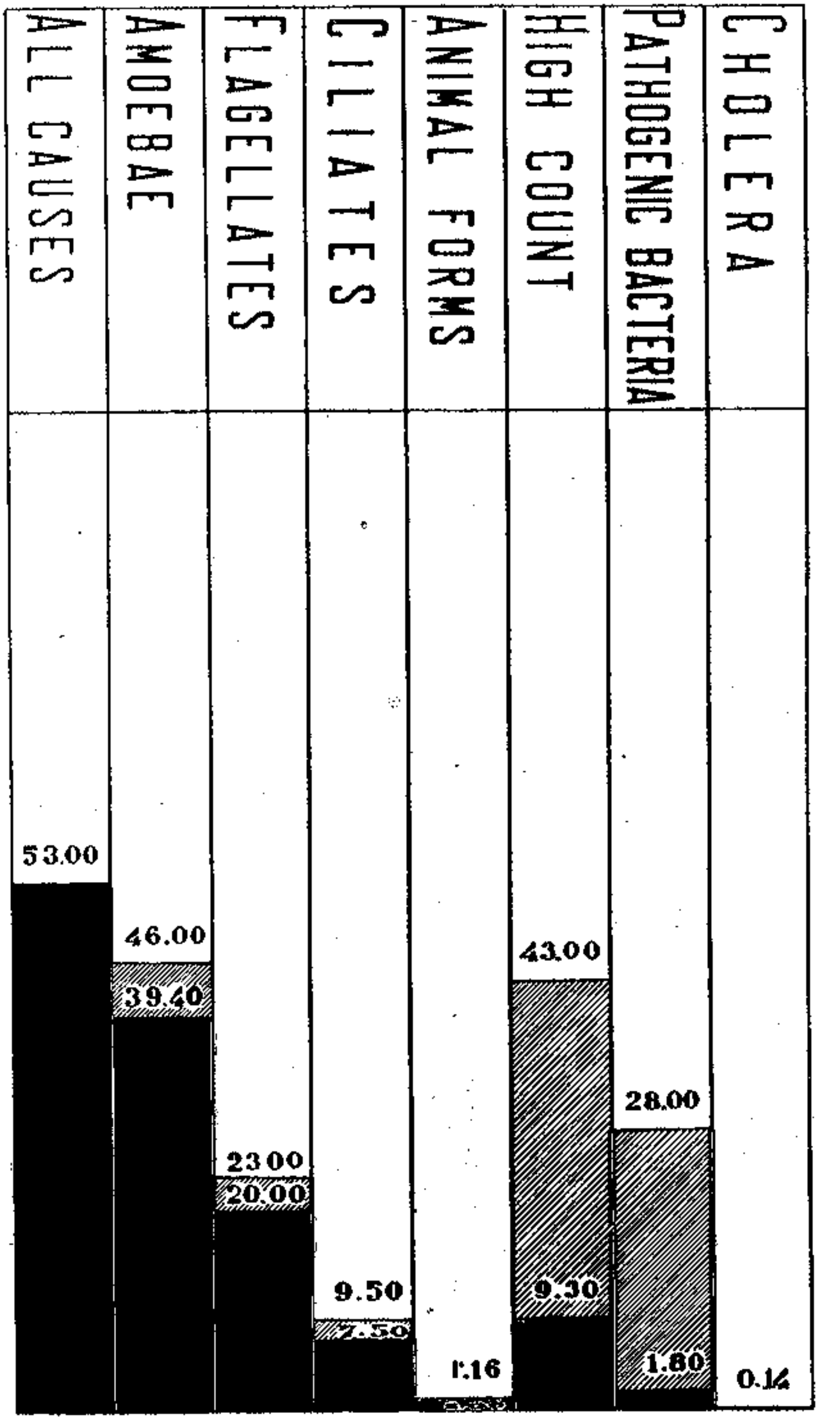
Waters bottled and for sale by reliable firms are safe. The danger of contamination of water with protozoa by handling it is great. This, coupled with the fact that we do not know absolutely what protozoa are pathogenic, makes the use for drinking purposes of all water handled in the ordinary ways, dangerous. All well, cistern, river and hydrant water should be efficiently sterilized and kept in sterile vessels until used.

Not enough careful work has been done on artesian water to justify any general conclusion, but it is safer than any other which is used without being sterilized: it having been repeatedly examined with negative results.

B. cabildonis is described as a new pathogenic species.

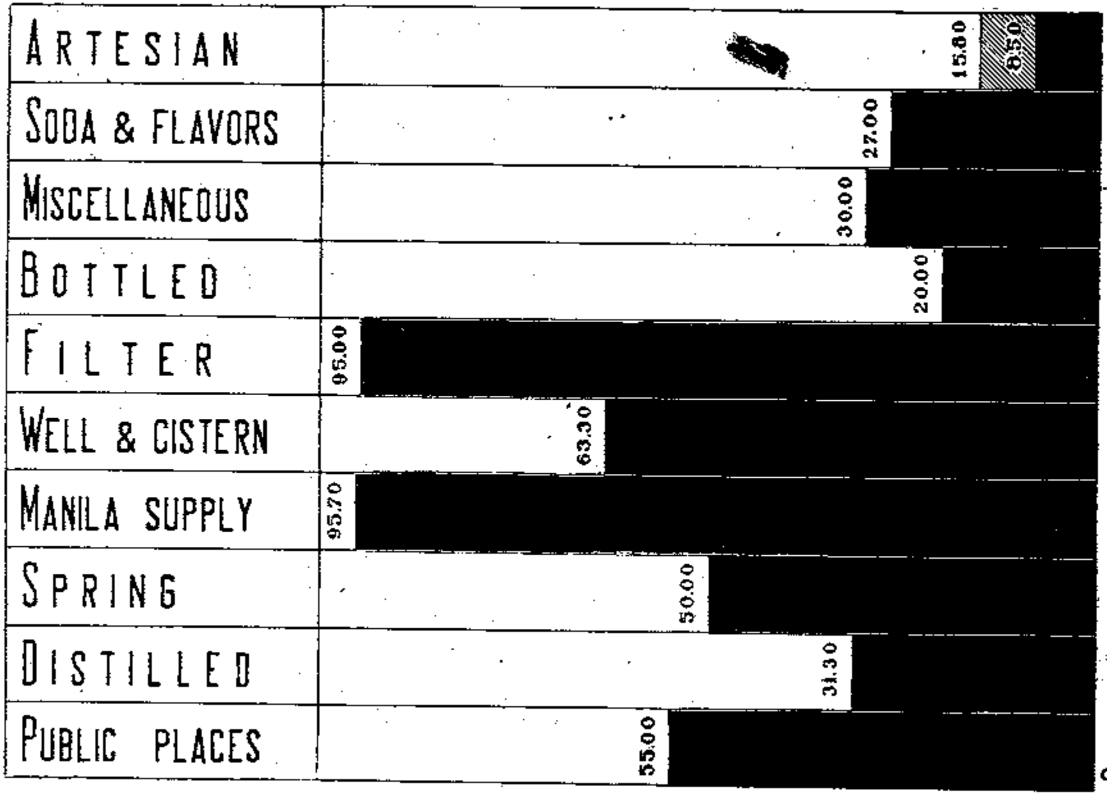
ILLUSTRATIONS.

Charts I and II.



PERCENTAGE OF WATER CONDEMNED FOR:

CHART I.



CONDEMNED PERCENTAGE OF WATER OBTAINED FROM

CHART II.

TYPHUS FEVER IN INDO-CHINA.

By A. YERSIN and J. J. VASSAL.[1]
(From the Pasteur Institute of Nhatrang, Annam.)

I.

On May 4, 1906, a thousand or so Tonquin coolies were landed at Nhatrang to serve on the railway in course of construction between Phanrang and Nhatrang. In spite of their youth they were men who had suffered, and they brought with them the poverty and the disease of their country. They were therefore predisposed to catch local endemics, especially malaria, but at the same time they showed affections which are perhaps limited to Tonquin and which in any case have not yet penetrated into South Annam. That recurrent fever came in this way, has already been made known by one of us; typhus fever will now receive our attention.

Our observations are not numerous, and if we have delayed their publication it is because we hoped to supplement them with new ones, but the Tonquinese handicraftsmen have disappeared from our district, and with them the means of pursuing a study which now could be undertaken again more easily in Tonquin.

Nevertheless, our experimental investigations throw new light on the pathogeny of typhus fever and leave no doubt as to the diagnosis of the cases observed in Nhatrang. If writers have dwelt at length on the distribution of typhus fever in Europe, records of its extension in Asia are brief. It exists in Persia, China, and Japan (Netter), but it seems to have attracted but little attention in India. It has never before been noted in the French possessions in Indo-China.

II.

We have been able to follow out seven observations on seven Tonquinese working on the embankment at Hoatan Suoi-Giau, young men between 18 and 20 years old, coming from the Provinces Ninh-Binh, Nam-Dinh, and Ha-nam. Transported under bad sanitary conditions in a crowded and badly organized boat, they were at once obliged to give themselves up to hard and unfamiliar labor in surroundings to which they were not accustomed.

---

[1] Read at the Fifth Annual Meeting of the Philippine Islands Medical Association, February 28, 1908.

All the cases were observed a short time after their arrival, between May 25 and June 1. The disease disappeared on the spot, without spreading to the native population or the other coolies on the railway. Our observations included five cases of natural and two cases of experimental disease. We will review them briefly.

NATURAL INFECTION.

CASE I.—Hoan Hiet, 20 years of age, from the village of Coi-Tri (Province of Ninh-Binh, south Tonquin), entered the hospital on May 25, 1906, for sores on the legs. On June 1 his temperature rose to 38° C., on the 5th to 40° C. A large abscess was discovered at this time, which was immediately opened. The temperature fell, but rose abruptly twenty-four hours later; after a careful examination it was easy to see that it was not caused by the wound, but that a new affection had begun. Moreover, the sores healed very rapidly. A number of very peculiar symptoms appeared little by little with the pyrexia. The patient fell into a state of profound prostration, which was not stupor but rather a complete annihilation of his strength, accompanied by a nervous upset. He was at times indifferent and dejected, at others delirious and agitated, his attitude was not without analogy to that shown by patients with bubonic plague. The patient complained and groaned continually. There was headache and insomnia. On the third day nausea began and the tongue remained coated without becoming dry. Constipation persisted during the entire illness; the abdomen was neither distended nor painful. The cardio-pulmonary apparatus revealed nothing abnormal on percussion or auscultation, although the breathing was accelerated and the pulse often as high as 120. There was no eruption on the skin nor on the mucosa, but the conjuctiva became considerably injected.

On the sixth and ninth days of the illness, the patient seemed more prostrated than ever, but without ceasing to complain; the prognosis became more serious. Suddenly, on the tenth day, a true crisis set in. The fever fell, the appetite returned and cerebral calm was restored. From that moment recovery was certain and continued without complication and with marvelous rapidity.

The temperature chart was high during ten consecutive days, ranging between 38°.2 and 39°.6 C. during the first eight days, and between 37°.9 and 38° C. on the two last ones. There were only three remissions in the morning between 37°.8 C. The defervescence which had been perceptible on the ninth day was sharply accentuated on the tenth.

CASE II.—Ngo Khoat, of the village of Coi-Tri (Province of Ninh-Binh, south Tonquin), from the same village as *Case I*, entered the hospital the same day as the latter.

The man had been found on the railway embankment already in full pyrexia. He was in a state of great weakness, the conjunctivæ very much injected; no eruption, and no intestinal phenomena were observed; he had been taken ill very suddenly five or six days before. In the hospital, the same principal phenomena were observed as in the preceding case. There was nervous disorder, cephalagia, delirium succeeded by prostration, habitual insomnia and tremor. The fever fell by crisis on the sixth day of his hospital treatment, which was probably the eleventh or twelfth day of his illness. There was a very rapid improvement and recovery without complications.

In spite of careful observation we did not find the least sign of eruption. Both Cases I and II received quinine in large doses by mouth and subcutaneous injection without result. The analysis of the blood, moreover, showed no signs of specific hæmatozoa.

CASE III.—Pham Khang, 28 years of age, also from the village of Coi-Tri, was ill several days on the railway works. We witnessed in the hospital only the terminal crisis which developed with remarkable suddenness. From the inquiry we made among the comrades of Pham Khang, we elicited the information that the following symptoms had been noted: Bloodshot eyes, prostration, fever without apparent remissions, constipation, rambling and incoherent complaints. The patient left the hospital on June 5, cured. An examination of the blood proved negative.

CASE IV.—Lai van Duc, 26 years of age, from the village of Thuong-Huu (Province of Nam-Dinh, south Tonquin), came into hospital on the second or third day of his illness, which had begun on the railway. We observed six consecutive days of very high fever, reaching 39°.9 and 40° C. with two matutinal remissions to 38.°4 and 38° C. The fall was abrupt; a slight ascent was noted the next day to 37°.4, then a descent to a point well below the normal during four days.

The same phenomena were present as in the preceding cases, except that diarrhœa followed several days of great constipation. No spots of any sort were present, but the conjunctivæ were again excessively red and inflamed. Quinine had no effect and there were no hæmatozoa in the blood.

CASE V.—Tran Luan, 24 years of age, from the village of Van-Khau (Province of Nam-Dinh, south Tonquin), entered the hospital on June 1, 1906, in the evening. On one of our weekly visits to the railway embankment, we found him very much prostrated and not able to stand alone. We took him into the automobile, but he was unable to remain in a sitting position without help. We learned that he had suffered from a sudden attack of fever the day before. His illness in hospital lasted ten days, the fever remaining high during six days. On the seventh there was a matutinal remission to 37° C. but the same day it again went up to 39°.3 C. in the evening. The next day there was a marked defervescence, and forty-eight hours later a return to normal, however, with a tendency to hypothermia during several consecutive days. Thus this case resembled those of the other patients. The clinical signs were reproduced with remarkable precision.

Let us repeat once more the characteristic features of this case. First, the sudden attack, fever lasting eleven days, great weakness with delirium, the miserable and distressing aspect of the patient, and lastly the rapid recovery after the crisis, and entire absence of relapse or complications.

Quinine medication was inefficacious and all the examinations of the blood were negative.

EXPERIMENTAL INFECTION.

We had tried in vain to reproduce the disease in the laboratory animals, such as rats, guinea pigs, or rabbits, and we thought that it would be of great scientific interest if we could be enlightened as to the transmission of the disease in the quickest possible manner, namely, by experiments on man. The relatively benignity of this cyclic fever justified this procedure. The positive knowledge of the nature of yellow fever and of its prophylaxis only dates from the first experiments on man

(Reed, Carroll, Agramonte and Lazaer, Marchoux, Salimbeni and Simond). With regard to dengue we have profited in the same manner during the last year (Ashburn and Craig).

CASE VI.—Nguyen van Ky, coming from the village of Nam-Dinh, Van-Khau (Province of Nam-Dinh, south Tonquin), was admitted into the native hospital for ulcers on the legs. His general state of health was excellent. Of robust constitution with a normal heart and without any symptoms of morbid diathesis, this man submitted voluntarily to an inoculation, of which the probable results were not unknown to him.

On June 1 Nguyen van Ky was inoculated under the skin of the arm with 0.50 gm. of blood taken from one of the veins of the arm of Tran Luan (Case V), who was in his second day's illness. The blood collected in a syringe, previously sterilized by steam, was immediately injected without the addition of any foreign substance. There was no reaction at the site of the inoculation. The lymphatic glands of the arm did not show the slightest modification.

The period of incubation lasted fourteen days. Suddenly, without any premonitory symptoms, the patient was seized with an intense fever which persisted for eleven days with slight variations, giving a most characteristic chart, identical in character with the ones taken from the natural illness. The temperature passed in one night from 35°.7 to 39°.5 C. The symptoms of the natural disease were reproduced with the greatest clearness and in addition congestion of the lungs was noted. The excessive prostration of the patient, his wandering thoughts, his insomnia, anorrhexia, coated tongue, constipation, the absence of intestinal phenomena, the suddenness of the attack and the rapid recovery after the crisis, were presented in detail. There was neither eruption nor spots on the skin, but the conjunctivæ were as usual congested.

As in the other cases, our microscopic researches were negative, and no specific febrifuge medication was successful.

CASE VII.—Tran Minh, 21 years of age, from the village of Do Le (Province of Ha-Nam, south Tonquin), entered the hospital on May 28, 1906, for fever and itch. His blood contained *Laverania malariæ*, and he had a series of irregular paroxysms which were checked by quinine. On the twentieth day in the hospital, when our patient had entirely recovered from his attacks of fever and when his blood contained no more hæmatozoa, we proposed to him that he should be inoculated with the blood of a typhus patient, at the time warning him of the probable consequences.

On June 20 he was inoculated under the skin of the arm with the blood of Nguyen van Ky (Case VI), who was in the fifth day of his experimental disease. The technique followed was the same as it was in the first instance. The amount of blood injected was 0.50 gm. and it was absolutely pure. The incubation period upon this second passage was longer than it was with the first patient, it being twenty-one days instead of fourteen. There was no local reaction.

As is seen from the chart, the temperature rose abruptly and remained at a high level for twelve days. It was impossible not to recognize the almost mathematically exact repetition not only of the fever, but of all the other symptoms. We again observed the identical signs; i. e., the abrupt beginning and end, the great weakness, the plague-like delirium, the constipation, insomnia, absence of eruption, blood-shot conjuctivæ, the inefficacy of quinine, and the absence of blood parasites.

III.

The diagnosis of the cases which have been cited was not immediately apparent. We had already observed some cases of spirillar fever among our Tonquinese patients, and therefore we thought ourselves justified in the supposition that we were again dealing with spirillar fever, presenting modified symptoms in the natives of south Annam. However, we hesitated to attribute a new disease, which had never previously been noticed, to the spirilla. The evidence soon became so positive that we were induced to think of typhus fever.

It is true, the eruption was wanting, and we tried in vain to obtain it in the experimental disease. Although failing in this, we succeeded in reproducing a peculiar, cyclic fever, a well-defined disease which corresponded exactly with the description of typhus fever. Although the actual exanthem failed in our cases, it is reasonable to suppose that the congested condition of the conjunctivæ was in a way a substitute.

This is the first time this disease has been described among the Annamese. Is it not possible that it has previously escaped observation because of the absence of an eruption? We know that in sporadic cases and in light ones the eruption generally fails. There have been epidemics without eruption (Dieulafoy), and all writers are agreed in pointing out cases without eruption in the most marked epidemics. Netter observes that "the eruptions in typhus fever are not constant; their absence is noted in a tenth or twentieth of all cases. These facts are not uncommon in the history of eruptive fevers. Dengue, for instance, causes epidemics where eruptions do not appear.

A diagnosis might also be made by elimination, after having reviewed the diseases which resemble typhus fever most closely.

The absence of Laveran's hæmatozoa from the peripheral blood and the spleen, the complete inefficacy of quinine, and the characteristic of the chart, permit us to set aside malaria.

The points of comparison with typhoid fever are very vague, but the differences are sharply accentuated. It is true that the tongue was coated, but it had not the dry and "roasted" appearance of that of typhoid fever; neither were there any intestinal phenomena, but on the contrary constipation. The abrupt start, the course of the chart and the crisis, differentiate our cases from typhoid or paratyphoid fevers.

Spirillar fever would not have passed unperceived at Nhatrang, where it had just been studied for the first time in Indo-China. This fever gives a chart with oscillations and relapses very different from that presented by the disease under discussion; besides, an examination of the blood settles the question.

Kala-Azar is too well known to-day to be confused with this disease.

Dengue is a disease that one meets with in Indo-China, sometimes well characterized, sometimes badly defined. But dengue lasts a still shorter time than typhus, and the charts are in no way comparable, any more than are the different symptoms.

Among the "nonclassified tropical fevers" Manson has described one which he calls "double continued fever," but this is removed from consideration in connection with our typhus by its relapsing pyrexia; and if we even regard the first attack only, the charts are in no way comparable.

IV.

Experiments were first tried on laboratory animals. A certain number of rats, guinea pigs, and rabbits received subcutaneously one or several inoculations of blood taken from patients at different periods of the disease. They manifested neither pathologic phenomena nor elevation of temperature. We have already related our experiments on man.

The examinations of the circulating blood as well as of specimens taken by splenic puncture, which were very numerous, were always negative.

These results agree entirely with those of Galesesco and Stalineano, in the typhus epidemic of Bucharest in 1906. Besides, we verified the fact as did these authors, that the terminal crisis is accompanied by a very marked and almost pathonomonic increase of mononuclear leucocytes. E. Gotschlich, on the contrary, has described protozoa resembling *P. bigeminum* in typhus patients at Alexandria, which he has called "apiosoma."

Our experiments prove that typhus fever is a disease in which the virus is situated in the circulating blood during the second and fifth days of infection.

It is inoculable from man to man, but does not seem to affect the ordinary laboratory animals. It is possible to make at least two passages on man, but it must be remarked that the incubation is longer the second time than the first (fourteen and twenty-one days).

The specific agent of typhus fever is invisible in the blood or at any rate is exceedingly rare.

These experiments, which doubtless require confirmation and elaboration, authorize us in considering typhus fever as a disease to be classed with the blood infections transmitted by the bites of insects. The transmitting agent has yet to be discovered. Medical literature on typhus, so rich in all kinds of epidemiologic documents, does not disprove this hypothesis.

V.

The therapeautic treatment ordinarily employed for malaria fails in typhus fever. Quinine particularly is useless. We limited ourselves to symptomatic medication.

The origin of our little localized epidemic must either be from the villages of our coolies or the boat which carried them. The infection may be developed later, but the germs seem to come from the south of Tonquin. The first three cases belonged to the same village, Coi Tri (Ninh Binh), and the other two to a bordering province, Nam Binh. The boat need not be considered, for if the disease had originated in it, the number of typhus cases would not have been so limited and there would certainly have been a focus on board, which could not have remained long unnoticed. Moreover, it was a Tonquinese vessel, the home port of which was Haiphong. Inquiry which will be made in the incriminated Tonquinese provinces will soon make it certain whether typhus fever has taken root in Tonquin, and whether new sanitary supervision will not be justified.

CONCLUSION.

1. Typhus fever has been observed in Annam in natives who recently arrived from Tonquin.
2. It manifested itself in accordance with the classic description, with the exception that the eruption was wanting.
3. The direct inoculation of blood from man to man has reproduced the disease after an incubation period of fourteen and twenty-one days.
4. Typhus fever seems to be a blood disease transmitted to man by bites of insects.

BIBLIOGRAPHY.

ASHBURN P. M. and CRAIG CHARLES F. Experimental investigations regarding the etiology of dengue fever, with a general consideration of the disease. *This Journ., Sec. B, Med. Sci.* (1907), **2**, 93.

G. DIEULAFOY. Manuel de Pathologie interne, Paris (1907).

GALESESCO et STALINEANO. Recherches bact. faites à l'occasion de l'épidémie de Typhus Exanthématique de Bucarest; Recherches cytologiques sur le sang, sur le liquide céphalo-rachidien dans le Typhus Ex. *Compt. rend. Soc. de biol.* Par. (1906), **61**, 15, 85, 230.

E. GOTSCHLICH (ALEXANDRIE). Ueber Protozoen befunde ("Apiosoma") in Blute von Flecktyphuskranken. *Deutsche med. Wchnsch.* (1903), **29**, 329–331.

MANSON, P. Tropical diseases: A Manual of the Diseases of Warm Climates. London (1904).

MARCHOUX, SALIMBENI et SIMOND. La fièvre jaune rapport de la Mission française. *Ann. d. l'inst. Pasteur* (1903), **17**, 665.

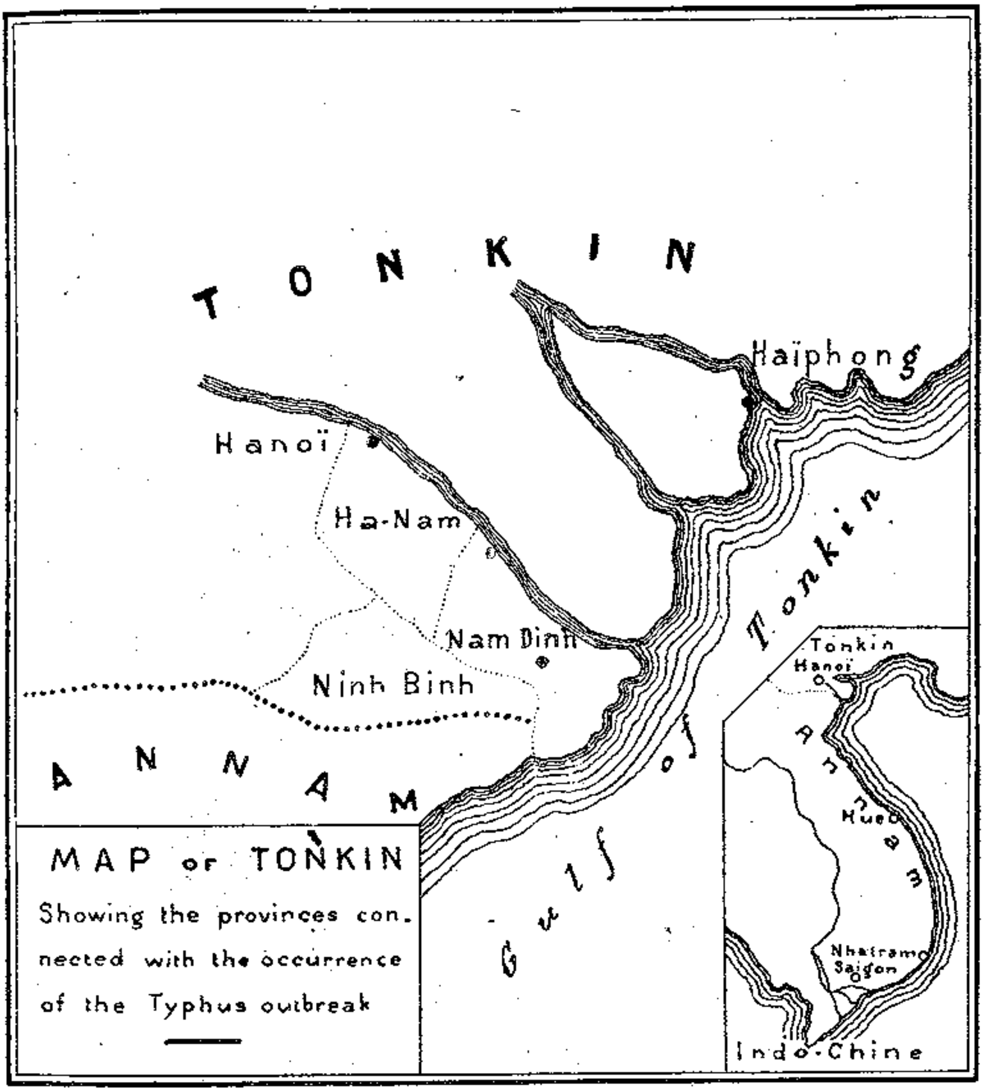
NETTER. Typhus Exanthématique, in Traité de Méd. et de Thérap. Brouardel, Gilbert et Girode: Paris (1896), **2**, 1.

REED, CARROLL, AGRAMONTE and LAZEAR. The etiology of yellow fever: A preliminary note. *Proc. Am. Pub. Health Asso. 28th an. meet.* Columbus (1901).

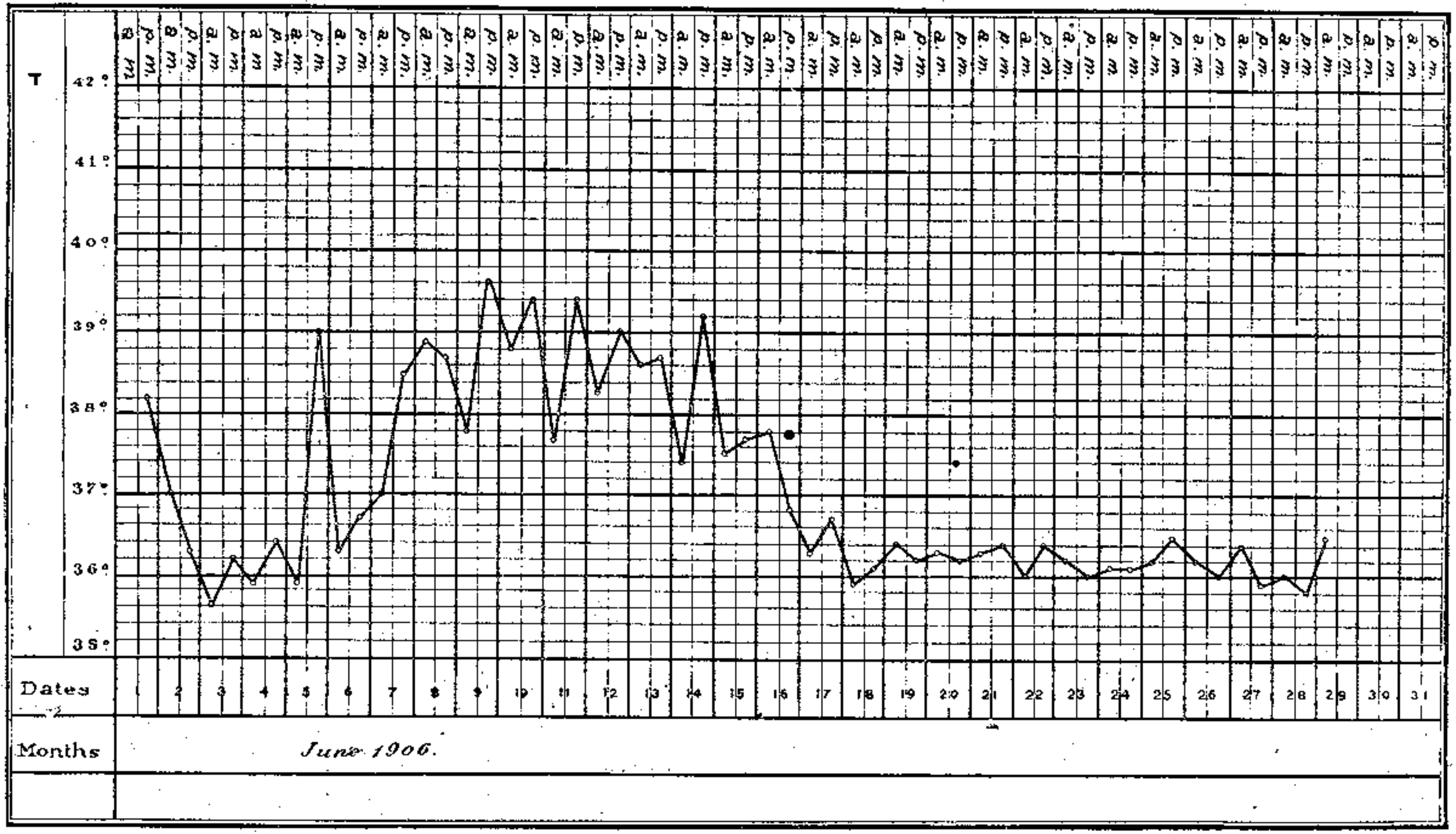
A. YERSIN. Note sur un cas de Fièvre Récurrente obs. en Indo-Chine. *Compt. rend. Soc. de biol.* Par. (1906), **60**, 1037.

ILLUSTRATIONS.

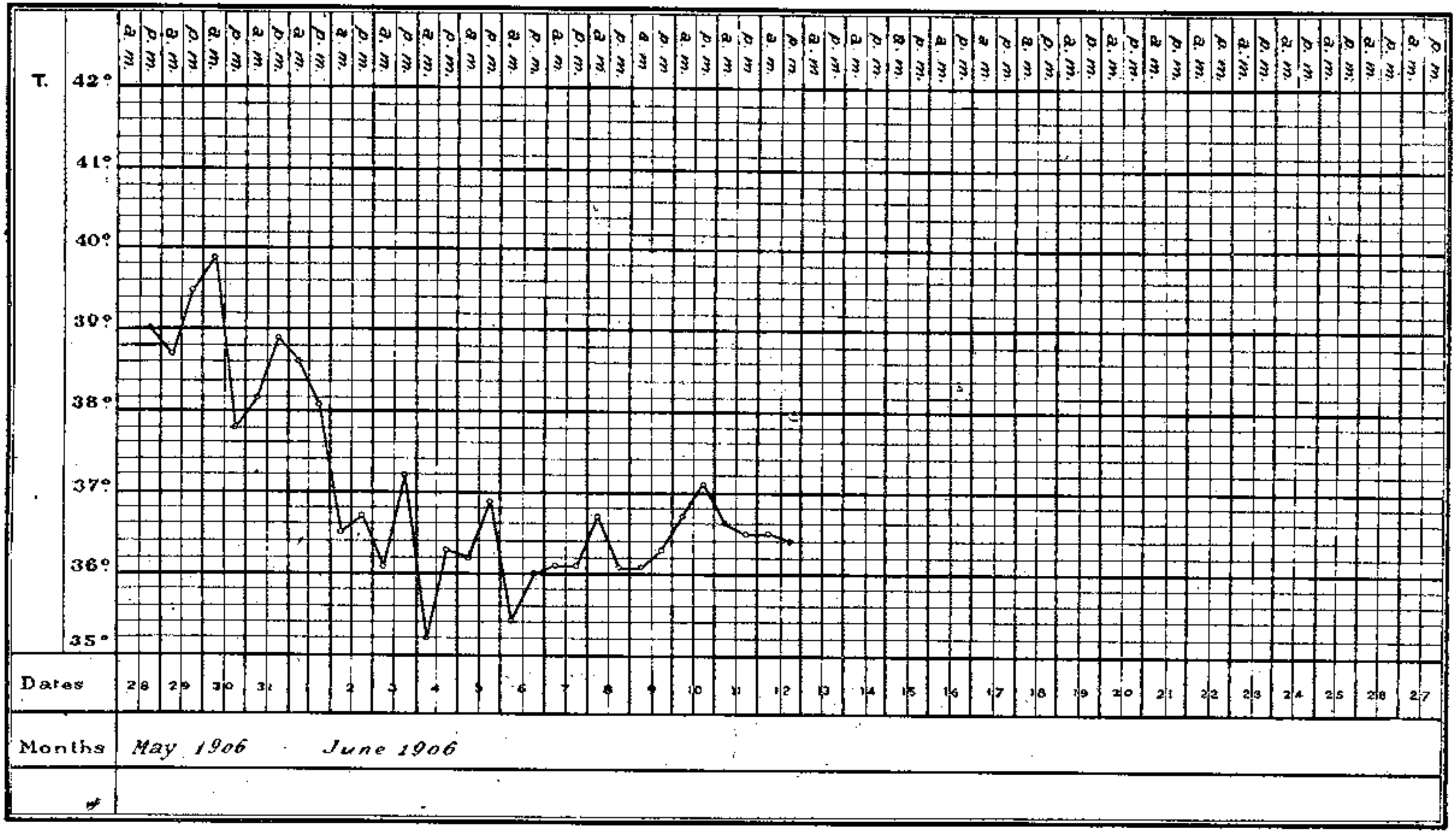
Map of Tonkin and Annam.
Charts 1 to 7.
139



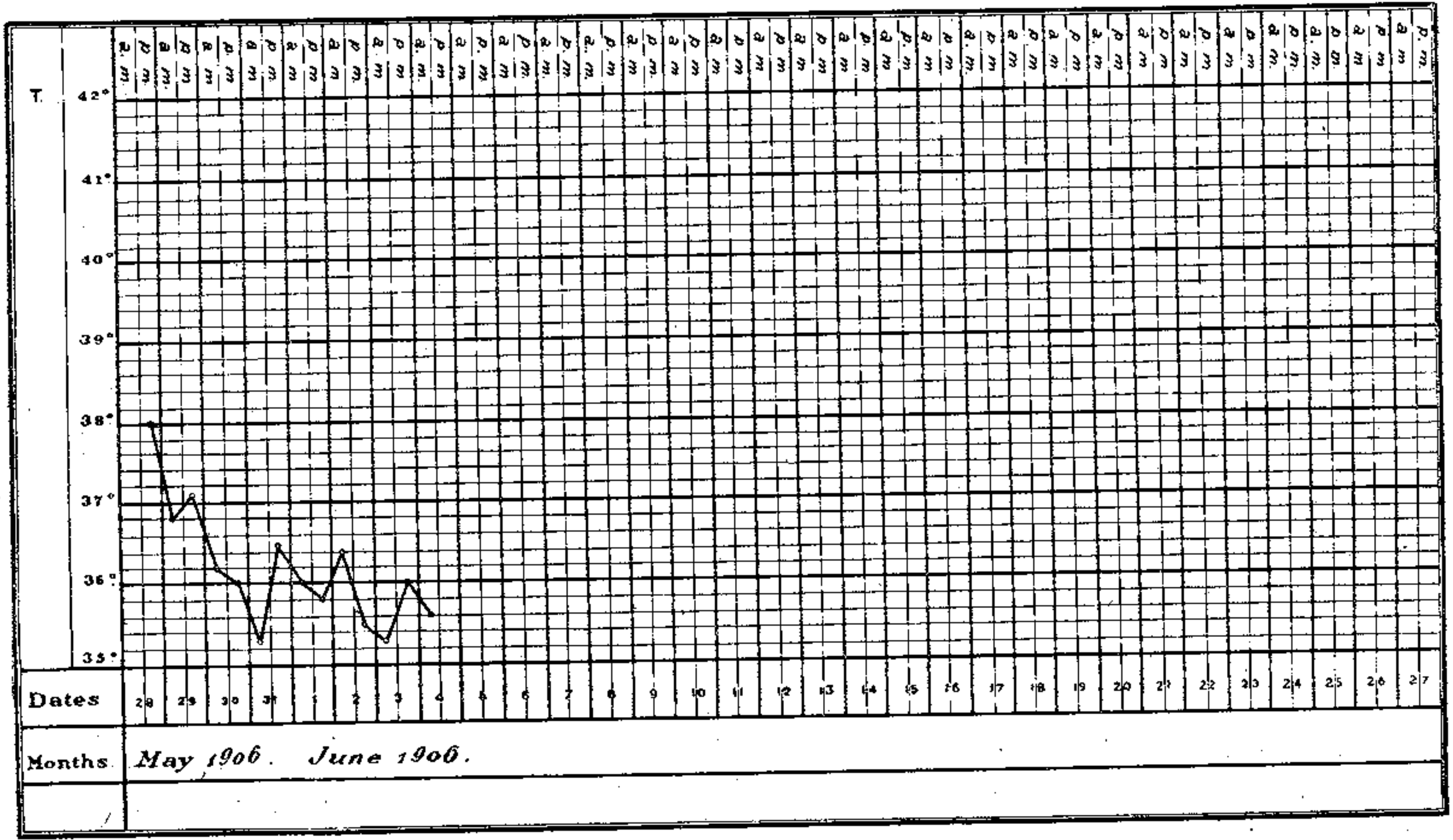
MAP OF TONKIN AND ANNAM.



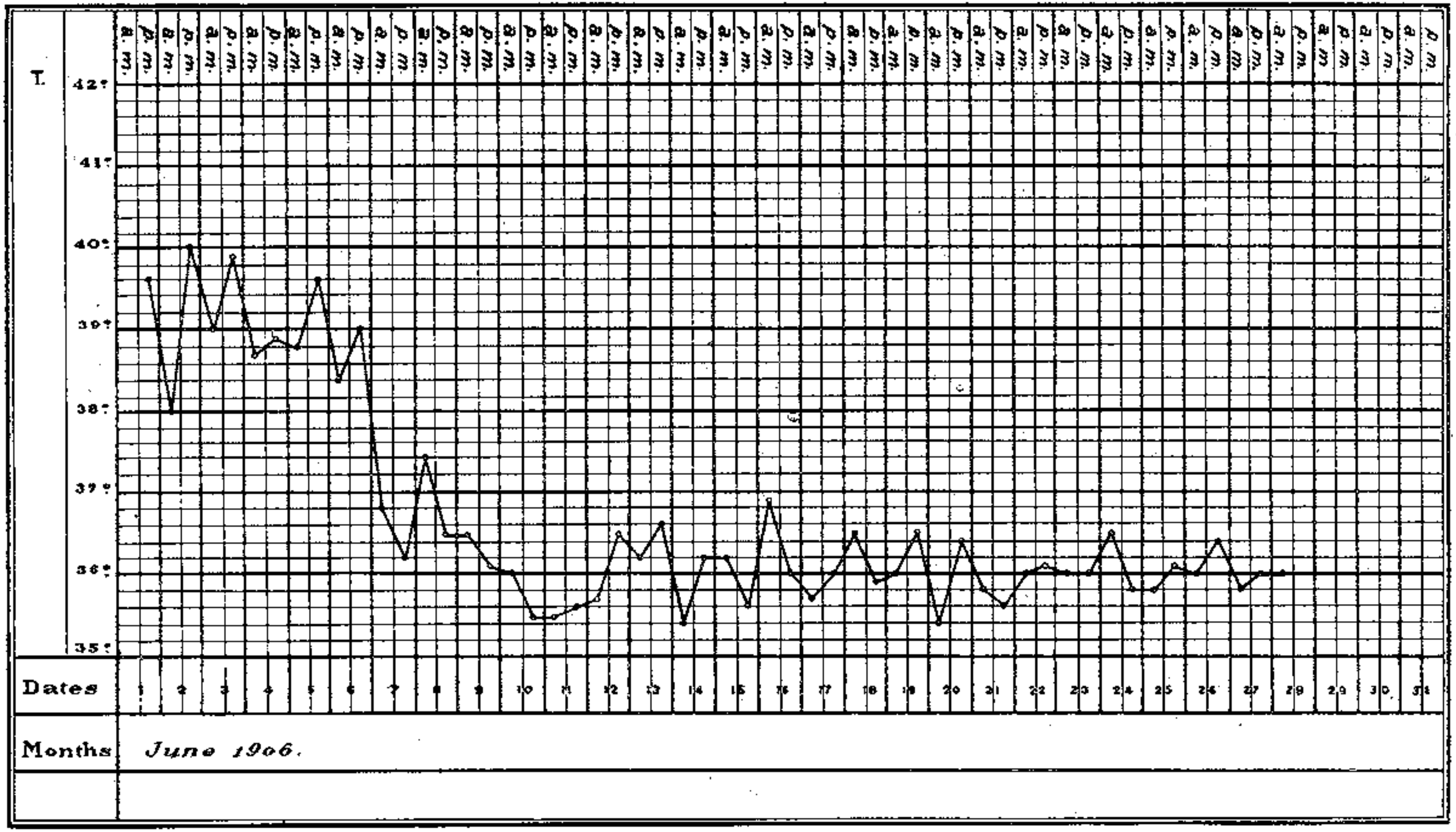
OBSERVATION 1.



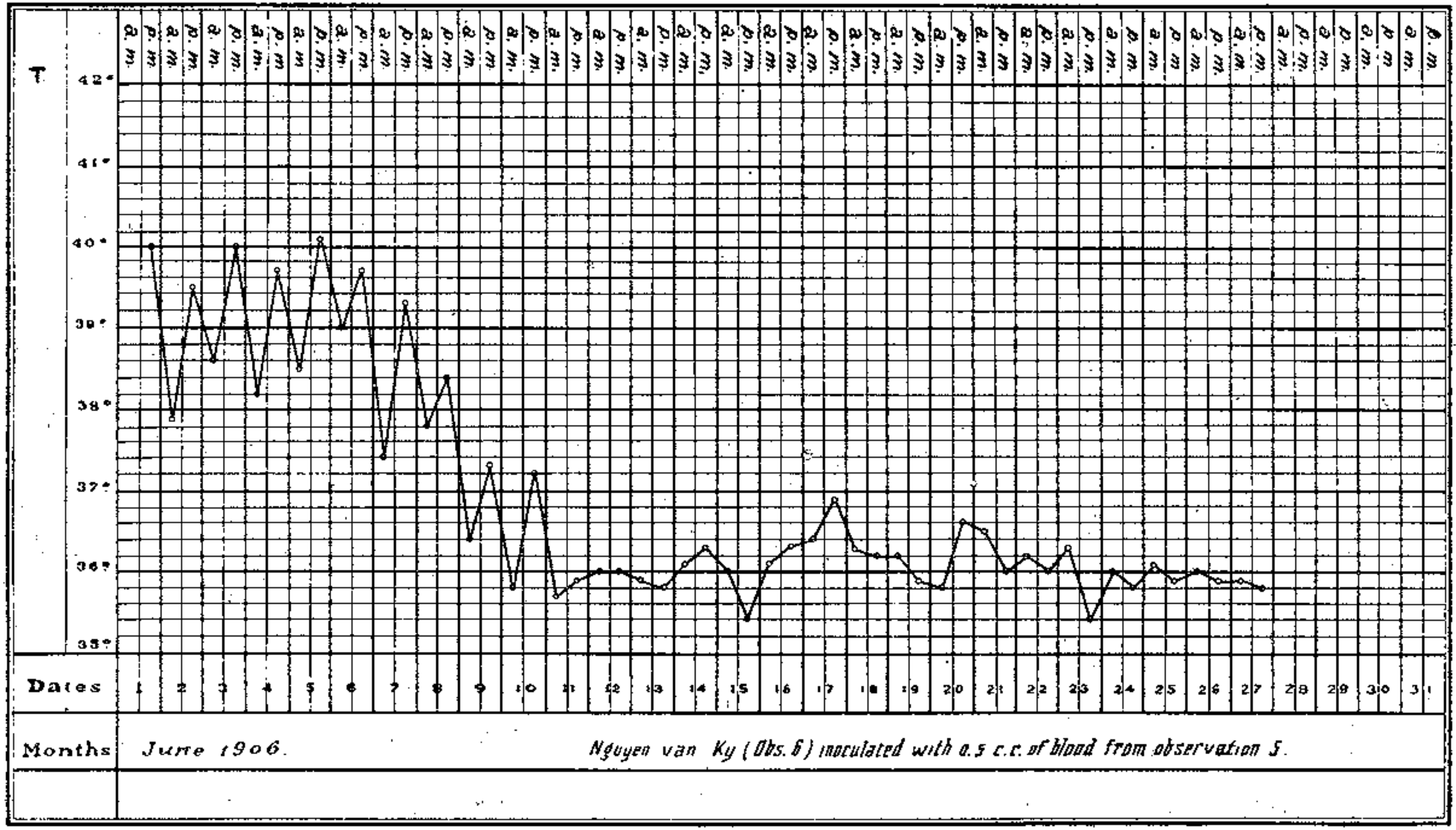
OBSERVATION 2.



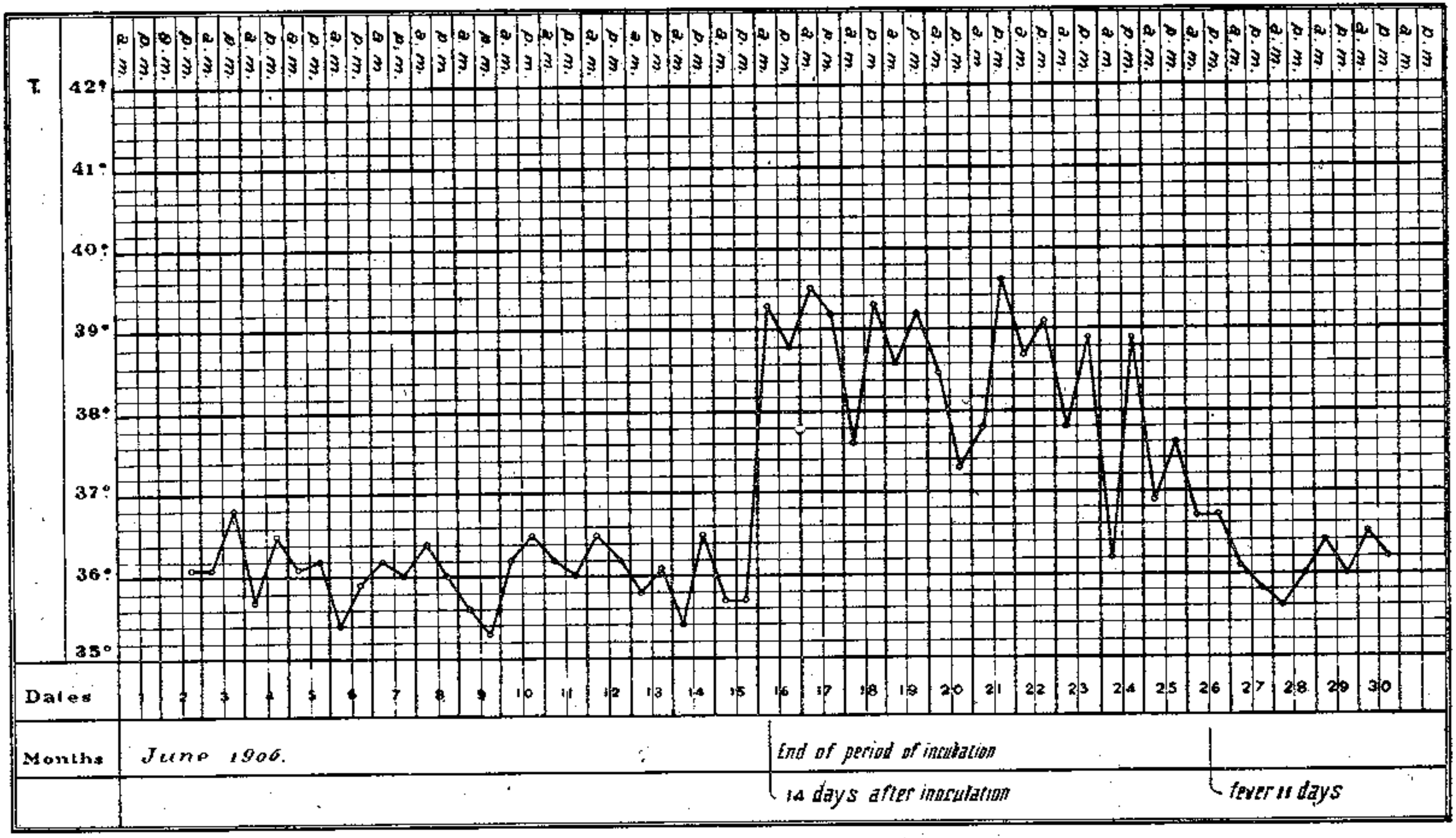
Observation 3.



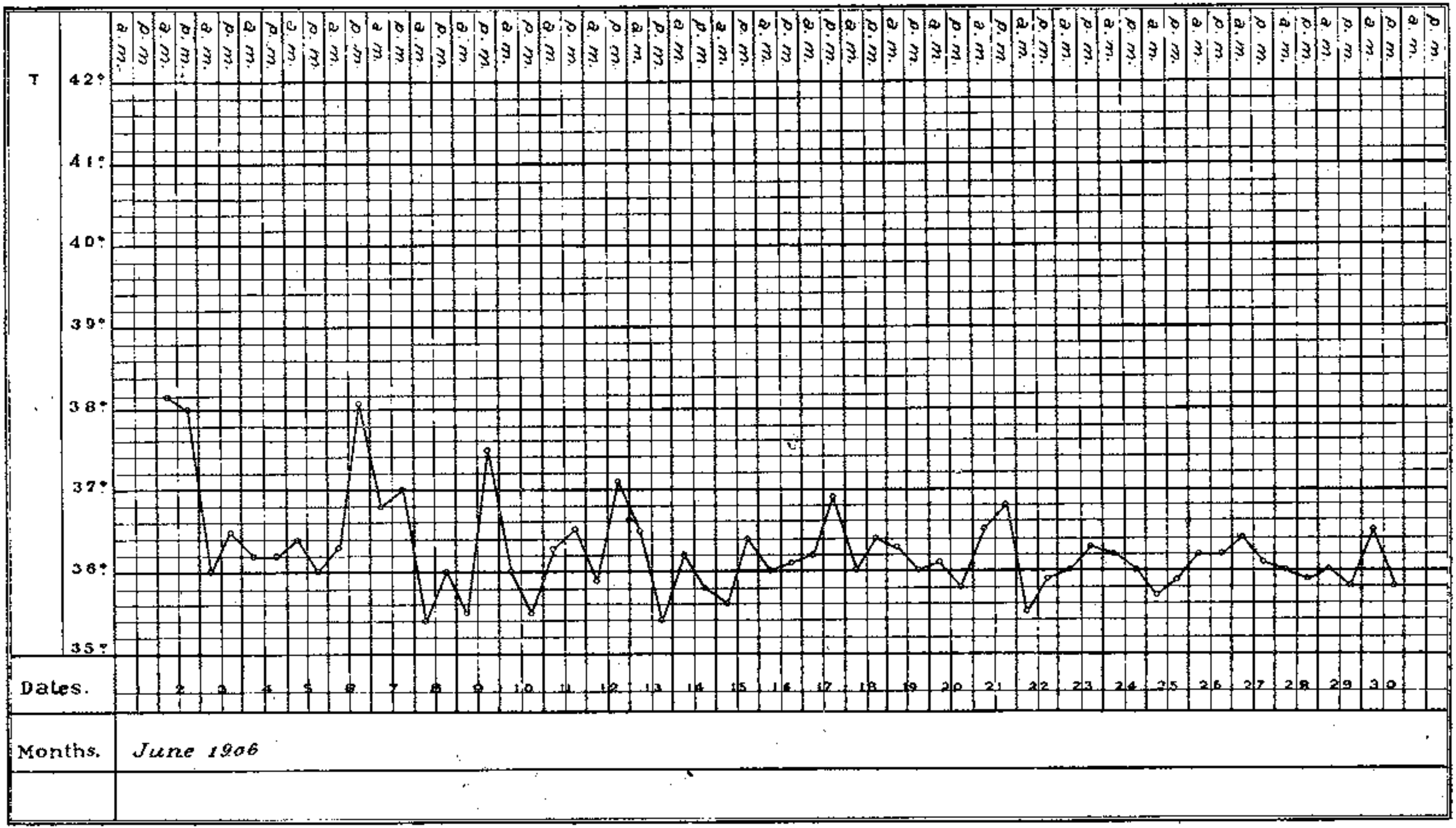
Observation 4.



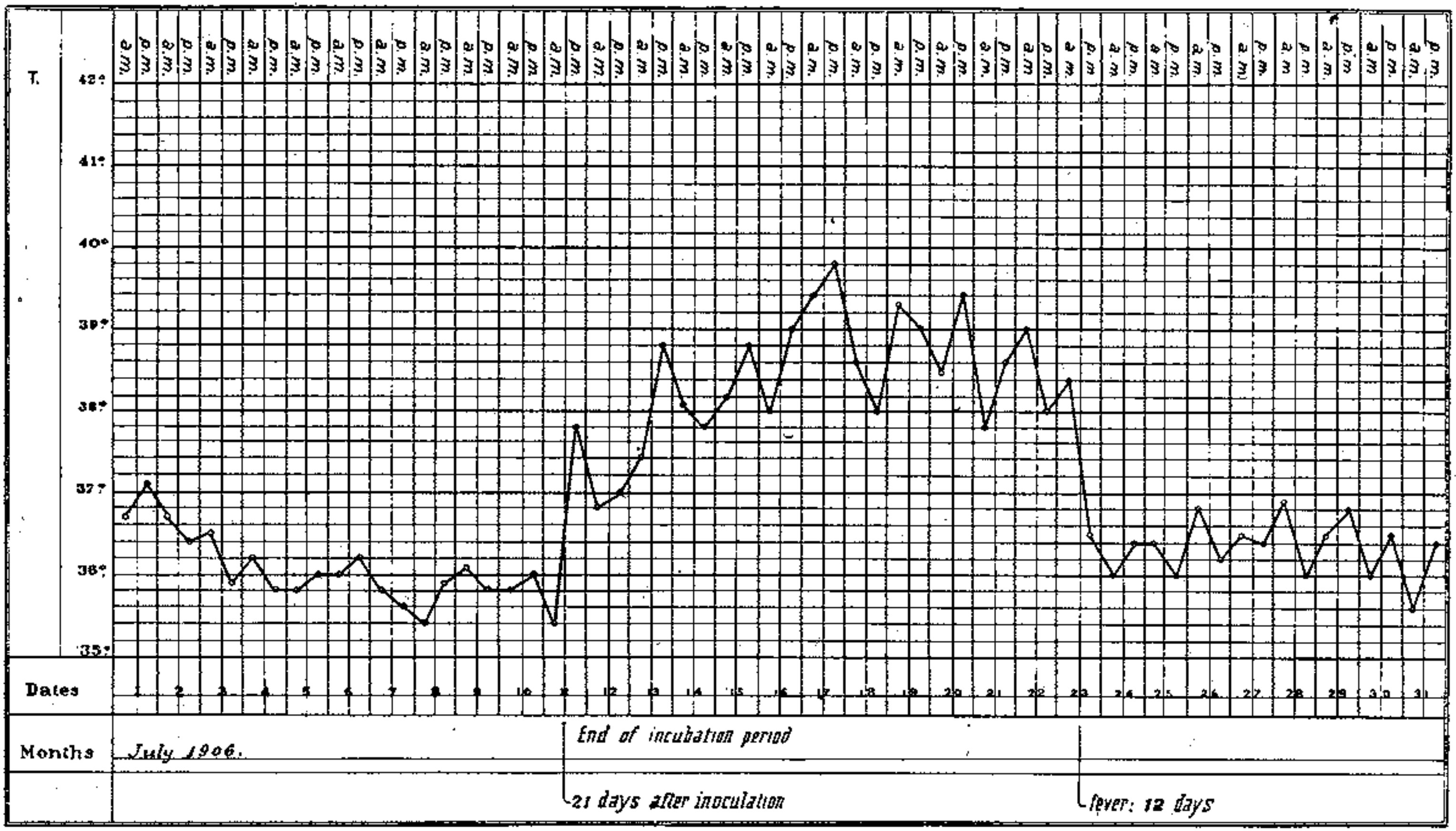
OBSERVATION 5.



Observation 6. June, 1906. Inoculated with 0.5 cc. of blood from Observation 5.



Observation 7. Inoculated June 20 with 0.5 cc. blood from Observation 6.



OBSERVATION 7—Continued.

ON "HABU" VENOM AND ITS SERUM THERAPY.

By T. Kitajima.[1]

(*From the Institute for Infectious Diseases, Tokyo, Japan.*)

INTRODUCTION.

Few species of venomous snakes are native to Japan, the main island, Nippon or Hondo, being inhabited only by the relatively harmless *Trigonocephalus Bromhoffi.* But the islands Riukiu (Loo Choo) and Amami (Oshima) are scourged by the fierce snake termed "habu." This species inflicts no little damage upon the population, either killing its victims outright or at least debilitating them seriously. The following table gives the number of cases occurring annually.

Table 1.—*Number of cases bitten by the venomous serpent "habu" from 1898 to 1906.*

Year.	Number of cases.			Recovered.			Deaths.			Infirmities.		
	Men.	Women.	Total.	Men.	Women.	Total.	Men.	Women.	Total.	Men.	Women.	Total.
1898	158	53	211	133	46	179	18	6	24	7	1	8
1899	149	64	213	121	53	174	22	10	32	3	1	4
1900	150	71	221	131	54	185	17	14	31	2	3	5
1901	148	83	231	130	68	198	18	14	32	------	1	1
1902	133	48	181	114	39	153	14	6	20	5	3	8
1903	172	71	243	140	57	197	24	4	28	11	7	18
1904	138	53	191	123	46	169	12	5	17	3	2	5
1905	182	79	261	153	63	216	27	11	38	5	2	7
1906	197	79	276	164	60	224	24	17	41	9	2	11
Total	1,427	601	2,028	1,209	486	1,695	176	87	263	45	22	67

This table shows that 2,028 persons were bitten by this serpent during the past nine years; of this number 263 died, an average of 225 cases with 29 deaths for each year, the death rate being 12.9 per cent. About the same number of cases has been reported from the Riukiu Islands; that is, about 200 a year.

---

[1] Read at the Fifth Annual Meeting of the Philippine Islands Medical Association, February 28, 1908.

I began the investigation upon which I am now reporting in the year 1902. A branch office of the Institute for Infectious Diseases was established and a temporary laboratory erected at Amami, Oshima, where I could have free access to the habu and so had facilities for collecting the venom. Since the time of beginning the work I have purchased more than 6,000 serpents. Dr. Mine, my former assistant, helped me in various portions of the research, and my present one, Yamamoto, is still engaged in collecting the venom at Oshima. I desire here to express my thanks for their collaboration and for the encouragement given by Professor Kitasato, director of the institute.

THE "HABU" SNAKE.

CROTALINÆ.

Trimeresurus flavoviridis (Hallowell).
Trimeresurus riukiuanus (Hilgendorf).

The length varies from 1 to 1.5 meters; the snake has a large, triangular, flat head which is separated from its spotted body by a distinctly marked neck. The fang is tube shaped. An indentation is present at the back of the eye.

The bites occur during the time from June to December, June and July being most dangerous months. No bitten persons have been reported in January and February. Eighty out of 174 cases were bitten on their lower limbs, 29 on the upper, and a few on the head, face, body, etc. It is dangerous to collect the venom, but finally in the laboratory we encountered a very dexterous manipulator among our employees. Even he would be bitten once or twice a year, but in the end the bites produced only a slight reaction, as he gradually acquired an immunity against the venom.

The process of collecting venom adopted by us is as follows: One of the snakes is taken out of the cage, its head quickly fixed with a forked stick, and as soon as the serpent stretches out its body on the floor its neck is caught with one hand and its mouth opened sufficiently to admit a watch glass, upon which the poison is discharged by compression from outside the sack at position of the poison gland. In our experiments the poison collected from a number of snakes was used for work.

HABU VENOM.

THE PHYSICAL AND CHEMICAL CONSTITUTION OF THE VENOM.

Venom collected from the habu is a viscous, slightly yellowish fluid with an acid reaction, 0.6 cubic centimeter of a 0.01 normal alkali solution being needed to neutralize 1 cubic centimeter. The quantity discharged at one time varies both with the size of the snake and the season of the year; it ranges from 0.5 cubic centimeter to 1 cubic centimeter; in rare cases it is 1.4. When the venom is centrifugated and the supernatant fluid desiccated, it forms a brilliant yellow powder. The amount of this fixed constituent is shown in the following table:

TABLE 2.

Locality from which snakes came.	Number of snakes.	Quantity of venom (cubic centimeters).	Dried (grams).	Fixed substance (per cent).	Average quantity of venom for one snake (cubic centimeter).	Average quantity dried (gram).
Tokunoshima	466	71.40	18.41	25.8	0.153	0.0395
Do	556	81.00	20.85	25.7	0.145	0.0375
Amani	195	58.47	16.46	28.2	0.299	0.0844
Do	418	122.10	27.29	22.4	0.292	0.0653

The dried venom is durable and it dissolves in water giving an opalescent solution with a slight precipitate; it is also almost completely soluble in glycerin, it coagulates at 75° C., and is precipitated by alcohol. It presents all the reactions characteristic of proteids, clearly showing Millon's and Vincent's reactions. It turns polarized rays to the left, and shows no lines of absorption in the spectrum.

Its chemical nature is not yet known clearly. The venom may be brought down by any of the reagents which precipitate proteids. About one-third of the original poison in a 1 per cent solution may be precipitated by adding alcohol to 33 per cent, and one-half of the whole amount of the poison present in the original solution from the filtered fluid will be precipitated by increasing the alcohol to 50 per cent. As more alcohol is added, the amount of precipitation increases, but the amount of the latter finally remains constant after the alcohol reaches 75 per cent. The greater part of the venom is precipitated if one-half the quantity of ammonium sulphate necessary to form a saturated solution is added.

RESISTANCE OF THE VENOM TO PHYSICAL AND CHEMICAL INFLUENCES.

The fact that the resistance of snake venom to heat seems to vary according to the species of snake from which it is collected has been ascertained by Mitchell and Reichert, Calmette, Physalix and Bertrand; Flexner and Noguchi, Lamb, and Ishizuka. Habu venom presents no change even though it is heated for thirty minutes at 60° C. A 1 per cent solution, however, will precipitate a little by being heated for fifteen minutes at 75° C., while its toxicity is reduced to one-tenth of the original. The principle which causes extravasation when the venom is injected is especially weakened, while the hæmolytic and neurotoxic constituents are scarcely affected. It loses nearly all toxicity if heated for thirty minutes at 90° C. The toxicity of the venom is reduced to one-tenth of the original strength by exposure to the direct sunlight during six hours.

Alkali has a far-reaching effect upon the venom, for when an equal volume of a one-tenth normal solution of sodium hydroxide is added, the toxicity falls to one-thirtieth of the original in twenty-four hours, while normal alkali solution completely inhibits the action of the toxin. The venom is also affected by acids. An equal volume of a one-tenth normal solution of sulphuric acid reduces the toxicity to one-fifth and a normal solution to one-tenth of the original strength after twenty-four hours.

The toxic character of the venom is destroyed by incubation for forty-eight hours at 37° C. with 1 per cent pepsin and 0.2 per cent hydrochloric acid; on the other hand there is a marked resistance to trypsin, which reduces the toxicity only one-half after forty-eight hours. Erepsin has been proved to have no influence over habu venom.

Snake venom is but little subject to the various changes affecting the diphtheria or tetanus toxins, it retains its toxicity unchanged for two months if it is dissolved in an 0.8 per cent salt solution to which 0.5 per cent carbolic acid has been added; however, in the end precipitation gradually sets in with a diminution of the toxicity. A solution in 50 per cent glycerin forms a transparent liquid showing no precipitation, and no diminution of toxicity. For this reason I have adopted the solution in 10 per cent glycerin as the standard and this, after dilution, was used in the usual experiments.

THE ACTION OF HABU VENOM UPON ANIMALS.

Habu venom is one of the most powerful poisons, affecting a very wide range of animals. Guinea pigs, rabbits, mice, rats, etc., which are commonly employed in experiments, die from inoculation of only a small quantity of it. However, its toxicity varies with the season during which it has been collected. The doses lethal after subcutaneous injection are as follows: For the mouse, 0.00005 to 0.0003; guinea pig, 0.0001 to 0.001; rabbit, 0.001 to 0.006.

The following figures are obtained according to von Behring's method by expressing the strength of the venom in terms of the body weight of animal killed by one gram of venom:

1 gram = 30,000 + Ms. to 200,000 + Ms.
1 gram = 500,000 + G. P. to 2,500,000 + G. P.
1 gram = 300,000 + R. to 2,000,000 + R.

or

1 gram = the fatal dose for 30,000 to 200,000 grams of mouse.
1 gram = the fatal dose for 500,000 to 2,500,000 grams of guinea pig.
1 gram = the fatal dose for 300,000 to 2,000,000 grams rabbit.

The above figures show the minimum lethal doses, killing rabbits and guinea pigs in twenty-four to thirty-six hours, and mice in less than twenty-four hours. If the death of the experimental animal takes more time than these fixed hours, it can hardly be relied upon; moreover, the suceptibility of the animal to the snake venom varies greatly in individuals, which makes the experiment more difficult than with the diphtheria toxin.

The differences in susceptibility of different species of animals to this poison is not so marked as it is to tetanus or diphtheria toxin.

The following table shows this, mice being taken as the standard:

TABLE 3.

1 fatal dose per gram of mouse=
    12 fatal doses per gram of guinea pig.
    10 fatal doses per gram of rabbit.
    10 fatal doses per gram of pigeon.
    5 fatal doses per gram of chicken.
    1 fatal doses per gram of dog.
    2 fatal doses per gram of rat.
    $\frac{1}{6}$ fatal dose per gram of cat.
    $\frac{1}{8}$ fatal dose per gram of frog.

It is evident from the above that the guinea pig and rabbit have the highest susceptibility, while frogs and cats have the lowest.

EXPERIMENTAL.

Mice.—The animals received 0.001 cubic centimeter of venom subcutaneously. The individuals appear ill after five minutes, with erection of the hair; slow, irregular respiration with inspiratory effort; paralysis of extremities causing difficult walking; a tumor is produced at the site of the injection, presenting a dark purple color. The tips of the ears, nose and claws become cyanotic; respiration at length becomes fainter and fainter, the pulse diminishes and is stopped in about forty-five minutes, when death takes place. Convulsions seldom appear.

Post-mortem examinations show a subcutaneous œdema with clots about the site of the injections. The liver, spleen and lungs are anæmic, their size being normal. If the dose used was sublethal, the animal gradually recovers from the affection, while the œdematous areas become necrotic and finally a spontaneous cure is effected at this site.

Guinea pigs.—The animals die in two hours after receiving 0.01 cubic centimeter subcutaneously; symptoms appear in fifteen minutes, swelling is evident at the site of the injection, it turns to a dark, purple color, spreading gradually; the abdomen becomes distended; the animal can not arise to its feet after being turned on its back; respiration becomes slow; the tips of the ears appear pale; shivering sets in, accompanied by a slight opisthotonus. Death takes place after paralysis has developed.

Post-mortem examination shows a subcutaneous œdema with hæmorrhage. The internal organs are anæmic without any other changes. The bowel contents are fluid.

Rabbits.—The animals die in five hours after receiving 0.03 cubic centimeter subcutaneously, practically with the same symptoms as are observed in other animals. A well-developed œdema at the site of injection makes its appearance, accompanied by hæmorrhage. The animal becomes so lethargic that it walks with difficulty and finally can not stand on its feet. An excessive dose, say of 0.04 cubic centimeter, when given intravenously, will kill a rabbit in three to ten minutes. The animal thus treated appears as if in severe pain, and dies after a period of irregular respiration, without any marked changes being observed upon post-mortem examination. If a dose which would kill a rabbit in an interval at some time between thirty minutes and several hours is given intravenously, symptoms identical with the ones which appear after subcutaneous injection are evident, and post-mortem examination shows clots in the pericardium, endocardium and in the mucous membrane of the stomach. Hæmaturia is seldom present.

Five one-hundredths of 1 per cent of the dose which is lethal in subcutaneous injections brings death to the animal when it is injected into the brain. In the latter event perturbation, agitation and convulsions occur and death is preceded by gasping. On post-mortem examination, clots are found at the site of the injection.

Intraperitoneal injection develops the same symptoms as the subcutaneous ones, the only difference being in the severe hyperæmia and ecchymosis of the bowel and other abdominal organs.

In the majority of instances the experimental animals recover gradually when the dose is sublethal. Only now and then death occurs secondarily accompanied by emaciation and conjunctivitis. Other animals die from septicæmia contracted by secondary infection through the ulceraton of the skin.

SYMPTOMS OF HABU POISONING IN MAN.

The region of the bite is of a dark, purple color and shows great swelling, accompanied by a severe burning pain, the latter being characteristic of habu venom intoxication. The lymph glands near the bite also become enlarged and sensitive to pressure. Loss of appetite and vomiting with colic and trouble in the thorax occur in chronic cases, the face is pale; the pulse feeble and rapid (90 to 120). In most instances respiration remains normal. A slight fever is not infrequent. The pupils are neither dilated nor contracted. A cold sweat ending in death finally sets in. The end is also preceded by coldness of the extremities and dyspnœa. Blood in the urine or in the fæces has been observed. Sensory or motor disturbances, which persists after recovery from acute symptoms, are usually due to secondary infections or improper treatment.

HÆMOLYSIS.

Habu venom consists of hæmorrhagic, neurotoxic and hæmolytic constituents. The hæmolytic action of various kinds of snake venom was observed by M. C. Martin in Sydney, by Calmette, Cunningham and others. Flexner and Noguchi,[2] Kyes,[3] and Ishizuka[4] have each published thorough investigations on the subject. They agree in respect to the complexity of the hæmolytic action of snake venom and find that this action is produced by the coöperation of complements and amboceptors. Kyes discovered that lecithin can act as complement in the hæmolysis produced by venom. The hæmolytic action varies with the different species of animals; our method of studying this phenomenon being as follows:

---

[2] *J. Exp. Med.* (1902), **6**, 277.

[3] *Berl. Klin. Wchnsch.* (1902), **39**, 886, 918.; *ibid.* (1903), **40**, 21, 57, 982.

[4] *Ztschr. f. exp. Path. u. Ther.* Heft 1, 4.

A certain amount of venom is mixed with a 5 per cent suspension of the defibrinated blood prepared with 0.8 to 0.9 per cent saline solution, and allowed to stand in an incubator for two hours. After this time it is left at the laboratory temperature for forty-eight hours longer, after which period the result is noted. The action at times would set in very slowly—occasionally not for two or three days. Of course, a control is to be made to distinguish the hæmolysis caused by the venom from the natural laking of the blood. The solution of venom must be prepared immediately before the experiment.

The following table shows the variation of the hæmolytic action with different species of animals:

TABLE 4.

One cubic centimeter of a 5 per cent suspension of blood cells from different animals shows minimal trace of hæmolysis with the following doses:

Dog	0.0000001
Cat	0.0000005
Horse	0.000001
Pig	0.000001
Hen	0.000001
Pigeon	0.000001
Guinea pig	0.00001
Rabbit	0.001
Man	0.0001

The quantities given in the above table are the minimum doses producing hæmolytic action, but five or ten times these amounts are necessary to secure complete laking. The blood corpuscles of cattle, goats and sheep are not affected.

The modification of the hæmolytic action by serum was studied, the following experimental method being used:

The blood corpuscles of the dog and other animals were washed four to eight times in saline solution, and divided into two parts, one of which was mixed with the serum of the same kind of animal and the other left free. Venom was then added to each series.

The following table shows the amount of venom required to produce hæmolysis with the serum-free corpuscles and with those mixed with serum:

TABLE 5.

Animal.	Without serum.	With serum.
	Gram.	*Gram.*
Dog	0.00001	0.0000001
Guinea pig	0.00002	0.00002
Rabbit	0.0001	0.0001
Horse	0.00005	0.000001
Pig	0.000001	0.000001
Pigeon	0.000001	0.000001
Hen	0.0001	0.00001

These figures make it evident that the presence of serum markedly promotes hæmolysis in the cases of the dog and the horse, has no influence in those of the guinea pig, rabbit, pig, pigeon and hen, while with the latter two birds the mixture of the serum even appears somewhat to retard the action.

Experiments were also undertaken to demonstrate whether the accelerator of hæmolysis offers as little resistance to heat as does complement in general, and for this purpose several portions of dog serum were heated for thirty minutes at 60°, 70°, and 80°C., respectively, and were then added to test tubes of washed dog's corpuscles mixed with varying amounts of venom. The resulting hæmolysis is noted in the following table:

TABLE 6.

Dose of venom (gram).	Serum not heated.	Serum heated to—		
		60° C.	70° C.	80° C.
0.00001	Complete	Complete	Complete	Complete.
0.000001	do	do	do	Do.
0.0000001	Partly	Partly	Partly	Partly.
0.00000001				

The hæmolytic action is therefore not affected by heat, even though the temperature be as high as 70° to 80°C. We conclude from this that the action of the serum is not due to ordinary unstable complement, however it may be produced by the presence of lecithin, as was found by Kyes, with cobra venom.

A series of experiments was also made to determine the maximum temperature which the hæmolysin of habu venom would endure. Tubes of a 1 per cent solution of the venom retained about one-tenth and one-thirtieth of the original power of the hæmolysin after being heated for thirty minutes at 60°C. and 70°C., respectively. When heated for thirty minutes at 80°C. the remaining action was exceedingly slight, but still demonstrable. The hæmolysin was completely destroyed after heating for thirty minutes at 90°C. The result is partially shown in the following table, the experiment being made with the washed blood cells of the dog, both with and without serum:

TABLE 7.

Temperature at which a 1 per cent solution of the venom was heated for 30 minutes.	Doso (dried venom) needed in laking 1 cc. of the washed blood cells of dog.	
	With serum.	Without serum.
	Gram.	*Gram.*
60°	0.0000001	0.0001
70°	0.000002	0.0003
80°	0.00002	0.001
90°		
Not heated	0.0000001	0.00001

Although the hæmolytic action is diminished after the solution has been heated for thirty minutes at 60°C., it is interesting to note that this temperature affects only the venom acting on serum-free corpuscles, and the addition of serum restores the power completely. Therefore, the diminution in strength is of a superficial nature, only in part affecting the constitution of venom. Those specimens which were heated for thirty minutes at 70°C and 80°C. exhibited a similar tendency, although with progressive decrease of power. The venom was wholly destroyed at 90°C., after which treatment it failed to act even in the presence of serum. This phenomenon was shown most clearly when experiments were made with the blood corpuscles of the horse. The following table shows the hæmolysis produced in the blood corpuscles of a horse, with heated and unheated venom, the decimals representing the hæmolytic dose required to dissolve 1 cubic centimeter of a 5 per cent suspension of horse's red blood corpuscles.

TABLE 8.

Venom.	Hæmolytic dose of venom.	
	Without serum.	With serum.
	Gram.	*Gram.*
Heated for 30 minutes at 60° C	0.0003	0.000003
Not heated	0.000005	0.000001

The presence or absence of serum does not influence the action of venom upon the red blood corpuscles of guinea pigs, the strength of the venom gradually disappearing as the temperature is raised.

Lecithin was proved to play an important part on the hæmolytic action of habu venom, just as Kyes has observed it to act as an accelerator with other snake venoms.

Ishizuka also seems to have observed the same fact. The following table gives the results of our experiments with lecithin:

TABLE 9.

Dose of habu venom.	1 cc. of a 5 per cent suspension washed red blood cells of dog—	
	With 0.1 per cent of lecithin.	With no lecithin.
1.0 cc. of a 0.01 per cent solution	Complete hæmolysis	Complete hæmolysis.
0.5 cc. of a 0.01 per cent solution	do	Partial hæmolysis.
0.1 cc. of a 0.01 per cent solution	do	Slight hæmolysis.
0.5 cc. of 0.001 per cent solution	do	No hæmolysis.
0.3 cc. of 0.001 per cent solution	Partial	Do.
0.1 cc. of 0.001 per cent solution	do	Do.
0.7 cc. of 0.0001 per cent solution	do	Do.
0.5 cc. of 0.0001 per cent solution	do	Do.
0.3 cc. of 0.0001 per cent solution	Slight	Do.
0 1 cc. of 0.0001 per cent solution	do	Do.

The laking sets in distinctly and quickly in the presence of lecithin even when such a small quantity of venom as 0.01 part of the minimum dose required to effect hæmolysis is used—this being the lowest limit. The blood corpuscles of the guinea pig require 2×10^{-5} gram of venom to produce hæmolysis if lecithin is not added, while they will lake with 2×10^{-8} gram when 0.01 per cent of lecithin is added; i. e., 0.001 of the minimum dose is sufficient to effect the action. Again, the blood corpuscles of cattle do not hæmolyse with venom alone, but a laking was observed to occur with 1×10^{-7} gram of venom when lecithin was present. In every case, the degree of the hæmolytic action differs with the amount of lecithin used. For example, the blood corpuscles of cattle are not affected with 1×10^{-4} gram of venom even if 0.001 per cent of lecithin is added. The following table shows the hæmolytic dose of venom for 1 cubic centimeter of 5 per cent suspensions of corpuscles from various animals treated in each case with 0.01 per cent of lecithin:

TABLE 10.—*Dose of venom necessary to effect hæmolysis in 1 cubic centimeter of a 5 per cent suspension of washed blood cells + 0.01 per cent lecithin.*

Animal.	Dose.
	Gram.
Dog	0.0000001
Horse	0.0000003
Cattle	0.0000001
Guinea pig	0.0000001
Rabbit	0.000001

The venom heated for thirty minutes at 60° C. will effect a vivid hæmolysis when lecithin is added; that is, hæmolysis will make itself manifest in the blood corpuscles of the rabbit even with 0.0001 gram of the heated venom, whereas 0.003 gram is needed if no lecithin is present. The same holds true of the blood corpuscles of the horse and dog. However, the hæmolytic action with lecithin is less than that with serum. This is another instance of the difference of the action between lecithin and serum.

TABLE 11.—*Minimum doses of venom heated for thirty minutes at 60° C. which were needed to effect hæmolysis in 1 cubic centimeter of a 5 per cent suspension of blood cells.*

Animal.	With 1 cc. of a 0.1 per cent solution of lecithin.	Without lecithin.
	Gram.	*Gram.*
Rabbit	0.0001	0.003
Horse	0.0000003	0.0008
Cattle	0.000005	----------
Dog	0.00001	0.0001

Kyes and Sachs observed the fact that cholesterin inhibited hæmolysis, especially when lecithin is present. Ishizuka made the same observations with habu venom, but I was unable to verify this fact.

AGGLUTINATION.

Habu venom has an agglutinative constituent in addition to that causing hæmolysis. The agglutinative action of habu venom showed some variations dependent upon the species of animal employed. There is no parallel between hæmolytic and agglutinative action. The following table shows the amounts of venom required to agglutinate 1 cubic centimeter of various species of red blood corpucles:

TABLE 12.—*Venom required to agglutinate 1 cubic centimeter of a 5 per cent suspension of defibrinated blood cospuscles.*

Animal.	Amount.
	Gram.
Cat	0.000001
Rabbit	0.0005
Pig	0.0001
Cattle	0.0001
Guinea pig	0.0001
Dog	0.01
Pigeon	0.01
Horse	Negative.
Goat	----------

The agglutinin was destroyed by heating the solution for thirty minutes at 70°C., whereas the hæmolytic power was retained.

IMMUNIZATION AND IMMUNIZED SERUM.

Immunization with the venom of the family *Crotalidæ* is as a rule difficult to produce, and so it was found to be with the habu venom. I employed horses, cattle, goats, etc., for this purpose, and found that cattle could be made immune with comparative ease, the horse only with the utmost difficulty, several of the latter falling victims to the experiment. The venom was heated or treated with calcium hypochlorite or iodine trichloride in order to diminish the toxicity before introduction into the animal body, but simple dilution with water was also highly satisfactory. The diluted toxin was injected subcutaneously, intravenous inoculation being dangerous and often rapidly fatal to the animal. In most cases we began the inoculation with 0.00001 cubic centimeter of a 1 per cent solution, which was doubled during the first few injections and then increased by small degrees, till at last the dose reached 500 cubic centimeters. It took two years to effect immunization, for the snake venom tends to develop necroses and consequently the interval between injection must be made much wider than is usual with other toxins.

Calmette employs the following method to determine the efficacy of the serum:

> He first determines the dose of venom, which, when given intravenously, will kill a rabbit in from fifteen to twenty minutes, and this is inoculated intravenously fifteen minutes after the animal receives a certain amount of serum intravenously. If 1 cubic centimeter of the serum were needed to protect a rabbit of 2 kilograms' weight, the serum is said to contain 2,000 immunization units. However, I preferred to test the serum after the method of Erhlich and von Behring, mixing the toxin and serum before inoculation.

In order to determine the protective value of the serum, various amounts of the latter are added to tubes containing a 1 per cent solution of the venom. The mixture is allowed to stand for thirty minutes before inoculation. The dose contains ten times the minimum lethal dose for the animal. For instance, if a mouse is used, 0.05 cubic centimeter of a 1 per cent solution of venom should be injected in order to give the animal 0.0005 gram of venom, for the minimum lethal dose for the mouse is 0.00005 gram. This dose is first mixed with a certain amount of serum and then diluted with water so that the whole volume comes to 0.4 cubic centimeter before it is inoculated. The proportions used are as follows per 15 grams of mouse: 0.05 cubic centimeter of a 1 per cent solution of venom, 0.1 cubic centimeter serum, 0.25 cubic centimeter water.

For practical purposes, 0.5 cubic centimeter of a 1 per cent solution of venom, 1 cubic centimeter of serum and 2.5 cubic centimeters of water are mixed, the mixture is allowed to stand for thirty minutes, and 0.4 cubic centimeter of it is inoculated subcutaneously.

A certain interval of time is necessary to effect a complete neutralization of venom with serum. If the mixture is injected immediately after it is prepared, the free venom will often kill the animal and therefore, in every case it is allowed to stand for thirty minutes. Even then, the results obtained are not perfectly uniform, for occasionally an animal receiving a large quantity of serum dies, while the one which receives less serum survives. This is due to the wide differences in individual susceptibility. Therefore, the standardization of the serum should be repeated over and over again. I employed mice or guinea pigs in the experiment, because the abundant supply of these animals gives facilities for making a wide series of experiments with a great number of animals. Five one-hundredths cubic centimeter of the immune serum prepared by me neutralizes 0.05 cubic centimeter of a 1 per cent solution of venom; that is, a certain quantity of serum neutralizes the same volume of a 1 per cent solution of venom. Sometimes serum with double this power was obtained but it was exceptional. I found that antiserum prepared by Calmette had no effect on habu venom, while ten immunization units of antitetanus serum could neutralize 0.0001 gram of venom. Again, the antihabu serum was observed to have no power over the poison of the common Japanese viper.

THERAPY.

Antihabu serum has been tried upon a number of cases in Amami, Oshima, and Riukiu since 1905. In most cases the serum has been distributed among the practitioners of the islands gratis. They are required to report the result.

A habu discharges, under natural circumstances, 0.3 cubic centimeter to 0.5 cubic centimeter of poison, equaling about 0.1 gram of the dried venom. This dose will be neutralized by 10 cubic centimeter of serum; however, for curative uses, the dose must be increased to a considerable degree, as the venom is usually introduced several hours before the immune serum is injected. At present 40 cubic centimeters of serum are put in a bottle as a curative dose for one case. In a chronic case, twice the dose or more is used. The injection is made near the bite, which is incised slightly, washed and dressed in the usual manner.

One hundred and eighteen patients were treated with serum of whom five died, making a death rate of 4.2 per cent. Further, of these five deaths, one received 40 cubic centimeters of serum after three hours had elapsed after the bite had been received and the patient died in a few minutes after the injection was made. We may safely exclude this case from the above figures and then the death rate becomes as small as 3.4 per cent. Of the remaining four deaths, one case expired of weakness arising from loss of blood, another of pyæmia in six days, while another succumbed in nine days, and the remaining one died in sixteen hours.

Appended is a sample of the reports, which are made upon the printed form packed with each bottle of serum:

Result obtained by the administration of antihabu serum.

Patient:	
Residence	Kanehisa Village in Oshima.
Occupation	Peasant.
Name	Jiro Yamada.
Age	42.
When and where bitten	4 p. m. Dec. 5, 1905; Yaiyama in Kanehisa village.
Site of bite and appearance	At the middle of the right palm. The bite consists of several wounds as if stuck with needles; purplish in color; swelling considerably.
General symptoms	Swelling increased by degrees. Extended as far as the third of the upper limb; burning pain; glands swollen; painful on pressure; temperature, 39; pulse, 110; face pale; vomiting, colic, and restlessness developed.
Prognosis at the first examination	Bad.
Serum:	
First injection—	
Date and hour	7 p. m. Dec. 5, 1905.
Amount	40 cubic centimeters.
Second injection—	
Date and hour	10 p. m. Dec. 5.
Amount	40 cubic centimeters.
Total amount of serum injected	80 cubic centimeters.
Site of injection	
Other treatment if any	Dressed the wound with aseptic treatment; gave emulsion of digitalis.
Duration after injection was made	Amelioration of symptoms developed on the second day after inoculation; on third nearly cured, except necrosis at bite.
Result	Lived.
Remarks	
Physician:	
Name	T. Tamaru.
Address	Naka Kanehisa village in Oshima.

We have come to the following conclusion, drawn from all the reports we have received: The serum has a marked tendency to ameliorate the intoxication, bringing about a quicker recovery. If it is given immediately after the bite, swelling only makes its appearance, with only the slightest phenomena of intoxication. In ordinary cases, general symptoms such as vomiting, colic and pain soon disappear, pain at the site of the bite also decreases in two to three hours and the swelling subsides after serum treatment.

The importance of serum therapy is well shown by the fact that the death rate was reduced to one-third of the ordinary. However, we regret that the ignorance of the people as well as of the physicians of the islands and the difficulties of communication make the poor victims forego serum treatment in the general run of cases. Possibly no death will occur if the victim receives serum treatment within one hour after the bite.

In conclusion, I must acknowledge my indebtedness to Professor Kitasato, director of the Institute for the Study of Infectious Diseases, for promoting these studies.

FILTRATION EXPERIMENTS WITH VIRUS OF CATTLE PLAGUE.

By E. H. RUEDIGER.

(From the Serum Section of the Biological Laboratory, Bureau of Science.)

INTRODUCTION.

The question as to whether the virulent factor of the blood of animals sick with cattle plague will or will not pass the Berkefeld or Chamberland filter is still an open one, and therefore a legitimate subject for investigation. The following brief review of the literature will make this conclusion clear:

Semmer, in the year 1895[1] reported that the contagium of cattle plague does not pass through the Chamberland filter. No mention is made in the reference whether he used filter F or filter B. Nencki, Sieber and Wyznikiewicz[2] found that extracts made from the organs of an animal suffering from cattle plague transmitted the disease when injected under the skin of nonimmune animals. After having been filtered through either the Berkefeld or the Chamberland candles these extracts were entirely harmless. Kolle and Turner[3] state that cattle-plague blood or bile are rendered nonvirulent on being passed through either the Berkefeld or the Chamberland filter. In order to exclude the possibility of an intracorpuscular microbe which might not be free in the blood, they dissolved the red blood corpuscles by mixing defibrinated blood with 0.2 per cent solution of sodium chloride. Inoculation experiments with filtrates of laked blood gave identical results.

Nicolle and Adil-Bey[4] found that the virus of cattle plague does not pass through the Berkefeld nor through the Chamberland filter; one part of defibrinated blood having been mixed with nine parts of water. In a later report[5] these authors state if peritoneal fluid is used for experimental work the filtrates from the Berkefeld and from the Chamberland filters are frequently infectious. Yersin,[6] on repeating the experiments of Nicolle and Adil-Bey verified their results. He injected physiological salt solution into the peritoneal cavity of an animal sick with cattle plague; four hours later he collected the peritoneal fluid, filtered it and with the filtrate inoculated nonimmune cattle.

---

[1] *Deutsche Zeitschr. f Thiermedicin* (1895), **22**, 37.

[2] *Centrbl. f. Bakteriol. Orig.* (1898) **23**, 535.

[3] *Ztschr. f. Hyg. u. Infektionskrankh.* Leipzig (1898), **29**, 361.

[4] *Ann. d. l'inst. Pasteur* (1899), **13**, 323.

[5] *Ibid.* (1902), **16**, 56.

[6] *Ibid.* (1904), **18**, 429.

The results obtained showed that the virus passed through Chamberland filters marked F but not through those marked B.

Memmo, Martoglio and Adani[7] came to the conclusion that the virus of cattle plague passes through the Berkefeld filter, but is arrested by the Chamberland. These authors experimented with virulent blood diluted with physiologic salt solution and filtered under pressure equal to one-half an atmosphere.

Woolley[8] closes his experiences on filtration as follows: "Apparently to judge from these results the causative agent of the disease may or may not pass through the pores of the Pasteur-Chamberland filter according to the conditions surrounding the experiment." What these conditions are the author fails to mention.

Recently Todd[9] defibrinated virulent blood by whipping, diluted it by adding four times its volume of physiological salt solution (0.8 per cent), and divided it into two parts, part A and part B. Part A he passed through a large, close-pored Berkefeld filter as slowly as possible; part B through a large and very porous one as rapidly as he could. Filtration was carried out by suction from an ordinary laboratory water pump and the difference of pressure was less than one atmosphere.

Fifty cubic centimeters of each of these filtrates injected under the skin of cattle proved to be entirely harmless. Three control animals which were inoculated with one-fifth cubic centimeter of the same blood before it was filtered contracted typical cattle plague. Ten days after the inoculation with the filtrates he injected the same animals with 10 cubic centimeters of virulent blood; they all contracted cattle plague, showing that they were not immune to the disease.

In a second experiment, Todd introduced 40 cubic centimeters of citrated virulent blood into the interior chamber of a sterilized Chamberland filter marked F. After carefully closing the open end of the filter, he placed it in the peritoneal cavity of an animal from Cyprus. It remained well. Thirteen days later he inoculated the animal with virulent blood, it contracted cattle plague and died eight days later.

In closing his report Todd says: "Reviewing the work which has been published on this question and the results of the experiments cited above, one is led to the conclusion that there is insufficient evidence in favor of the active agent of cattle plague being capable of passing through a filter.

It will be seen from the foregoing that there is a wide discrepancy in the results of the different workers. Semmer, Nencki, Sieber and Wyznikiewicz, Kolle and Turner, and Todd agree that the virus of cattle plague will not pass any ordinary germ-proof filter. Memmo, Martoglio and Adani find that it passes through the Berkefeld filter but not through the Chamberland. Nicolle and Adil-Bey, as well as Yersin, state that it passes the Berkefeld and the Chamberland marked F, but is arrested by the Chamberland B. According to Woolley the virus may or may not pass the the pores of the Pasteur-Chamberland filter. Although there are wide discrepancies in the results reached by these authors, the distinctions are perhaps not greater than the differences in the methods employed by them.

[7] *Ann. d'Ig. Sper.* (1904), **14**, 291.

[8] *This Journal* (1906), **1**, 582.

[9] *J. of Hyg.* (1907), **7**, 570.

In carrying out the experiments to be detailed I made an attempt to repeat the various methods employed by different workers, and am able to report on the following:

I. Filtration of bile. II. Filtration of blood diluted with salt solution. III. Filtration of blood laked with distilled water. IV. Filtration of peritoneal fluid.

Throughout these tests great precautions were taken to prevent accidental infection of the experimental animals. In order fairly well to exclude the possibility of their having been accidentally infected before inoculation, the cattle were kept under observation in a fly-proof stable for at least seven days. If during this time no symptoms manifested themselves the animals were inoculated. Two control animals, A and B, were kept with each experiment. A received unfiltered material, while B remained uninoculated. Animal A was isolated in a fly-proof shed, animal B remained with those that were inoculated with filtrate. Animals that did not contract cattle plague from the filtrate were afterwards tested for immunity with virulent blood. The animals used were from Sibuyan Island as, so far, animals immune to cattle plague have not been encountered among cattle from that point.

EXPERIMENTAL.

No. 1.—The bile was collected and mixed with an equal part of physiological salt solution during the post-mortem examination on an animal bled to death for virulent blood. The mixture was divided into four parts, designated as *a*, *b*, *c*, and *d*. Part *a* was not filtered, *b* was passed through a Berkefeld filter V, *c* was filtered through a Berkefeld candle N, and *d* was passed through one marked W.

Four animals, Nos. 1, 2, 3, and 4 were inoculated.

Animal No. 1 received 50 cubic centimeters of part *a* under the skin. On the seventh day after the inoculation the temperature rose and the animal died of cattle plague on the thirteenth day. (See Chart No. 1.)

Animal No. 2 was injected with 50 cubic centimeters of *b*. Cattle plague did not follow. (See Chart No. 2.) Nineteen days later 10 cubic centimeters of virulent blood was given subcutaneously. In four days the animal sickened with cattle plague and was bled to death for virulent blood on the sixth day after inoculation. (See Chart No. 2′.)

Animal No. 3 received 50 cubic centimeters of *c*. It remained well. (See Chart No. 3.) Twenty days later it was inoculated with 10 cubic centimeters of virulent blood; it sickened with cattle plague three days later and was bled to death on the sixth day. (See Chart No. 3′.)

Animal No. 4 inoculated with 50 cubic centimeters of *d*, remained well. (See Chart No. 4.) After sixteen days it received 10 cubic centimeters of virulent blood, it sickened with cattle plague on the fourth day and was bled to death on the tenth after inoculation. (See Chart No. 4′).

No. 2.—Virulent blood was diluted with four times its volume of physiological salt solution and the mixture divided into four parts designated as *a*, *b*, *c*, and *d*.

Part *a* was not filtered, *b* was passed through a Berkefeld filter V, *c* was filtered through a Berkefeld candle marked N, and *d* was passed though one marked W.

Four bovines, Nos. 5, 6, 7, and 8 were inoculated with these materials.

Animal No. 5 received 50 cubic centimeters of *a*, it developed cattle plague on the fourth day after inoculation and was bled to death on the tenth. (See Chart No. 5.)

Animal No. 6 was inoculated with 50 cubic centimeters of *b* and remained well. (See Chart No. 6.) Twenty-eight days later the animal received 10 cubic centimeters of virulent blood, it showed symptoms of cattle plague on the third day and was bled to death on the sixth. (See Chart No. 6′.)

Animal No. 7 was injected subcutaneously with 50 cubic centimeters of *c*. No symptoms appeared. (See Chart No. 7.) Seventeen days later it was inoculated with 10 cubic centimeters of virulent blood, it developed cattle plague and was bled to death on the eighth day after inoculation. (See Chart No. 7′.)

Animal No. 8 received 50 cubic centimeters of *d*, it remained well. (See Chart No. 8.) After one month 10 cubic centimeters of virulent blood were injected, the temperature began to rise within twenty-four hours and the animal was found dead on the morning of the sixth day. (See Chart No. 8′.)

No. 3.—One part of virulent blood was laked in four parts of distilled water and the resulting solution was divided into four parts, *a*, *b*, *c*, and *d*.

Part *a* remained unfiltered, *b* was passed through a Berkefeld filter V, *c* was filtered through a Berkefeld candle N, and *d* was passed through a Berkefeld filter marked W.

Of four bovines, Nos. 9, 10, 11, and 12, animal No. 9 was inoculated with 50 cubic centimeters of *a*. The animal showed cattle plague on the third day after inoculation and was bled to death on the seventh. (See Chart No. 9.)

Animal No. 10 received 50 cubic centimeters of *b* under the skin. It remained well. (See Chart No. 10.) On the nineteenth day 10 cubic centimeters of virulent blood were injected, this procedure was followed by cattle plague in four days. (See Chart No. 10′.)

Animal No. 11 was inoculated with 50 cubic centimeters of *c*. This proved to be noninfectious. (See Chart No. 11.) Eighteen days later the same animal was inoculated with 10 cubic centimeters of virulent blood, it sickened with cattle plague in two days and was bled to death on the fifth day after inoculation. (See Chart No. 11′.)

Animal No. 12 received 50 cubic centimeters of *d* and remained well. (See chart No. 12.) After twenty days it was injected with 10 cubic centimeters of virulent blood. Cattle plague manifested itself on the fifth day and the animal was bled to death on the eighth. (See Chart No. 12′.)

No. 4.—This experiment is an attempt at repeating the work of Nicolle and Adil-Bey and that of Yersin.

Two liters of sterilized physiological salt solution were injected into the peritoneal cavity of an animal sick with cattle plague. One hour after the injection, the animal was bled to death, the abdomen opened and the peritoneal fluid collected. The collected fluid was diluted with an equal part of physiological salt solution and divided into four parts, *a, b, c,* and *d.* Part *a* was not passed through a filter, part *b* through a Berkefeld candle V, *c* was through a Berkefeld filter marked N, and *d* through one marked W.

Four bovines, Nos. 13, 14, 15 and 16 were inoculated with these fluids.

Animal No. 13 received 50 cubic centimeters of part *a,* it sickened with cattle plague on the third day and was bled to death on the sixth after inoculation. (See Chart No. 13.)

Animal No. 14, inoculated with 50 cubic centimeters of *b,* sickened with cattle plague within three days and was bled to death on the fifth day. (See Chart No. 14.)

Animal No. 15. Fifty cubic centimeters of *c* were injected into animal No. 15. Cattle plague appeared on the sixth day and the animal was bled to death on the tenth after inoculation. (See Chart No. 15.)

Animal No. 16 was inoculated with 50 cubic centimeters of *d,* it developed cattle plague within three days and was bled to death on the eighth. (See Chart No. 16.)

CONCLUSIONS.

According to the reports of other writers and the results above recorded, it appears that the causative agent of cattle plague present in the bile and in the blood of an animal sick with that disease does not pass through the pores of Berkefeld filters marked V, N, or W.

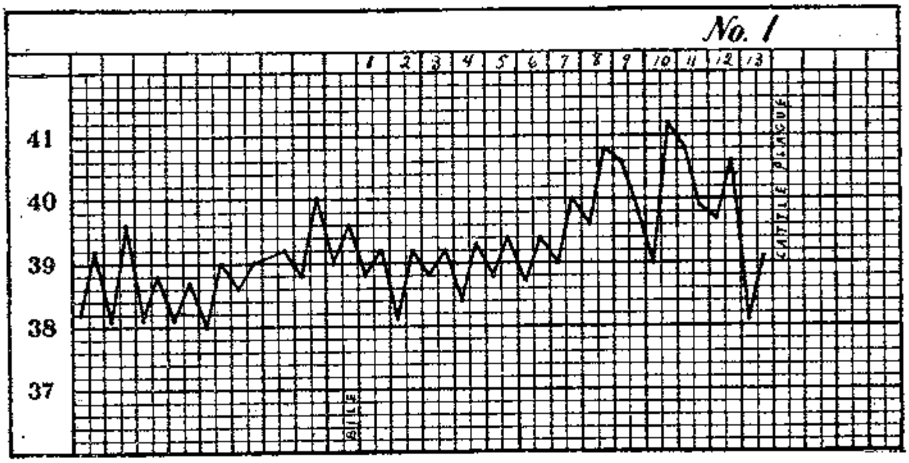
If one injects physiological salt solution into the peritoneal cavity of an animal suffering from cattle plague and collects the fluid one or two hours later, the fluid appears to be infectious after having been passed through either one of these three Berkefeld filters.

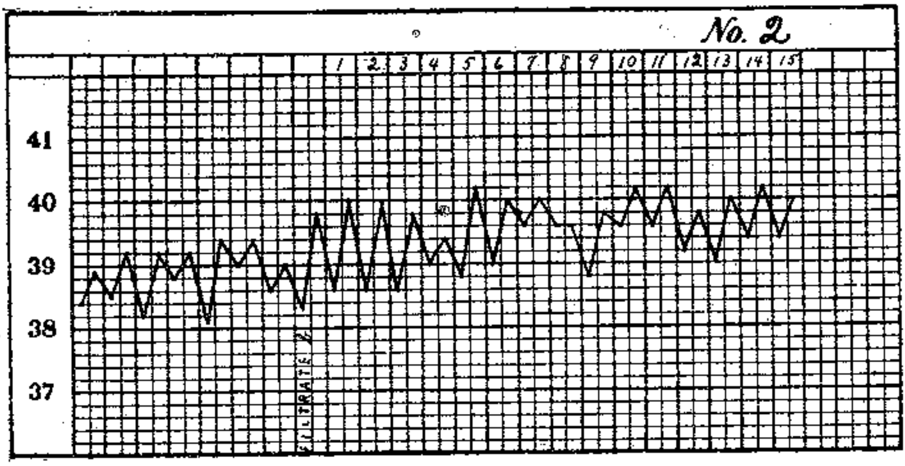
These results are somewhat unintelligible and the tests with peritoneal fluid will need to be repeated a number of times before accidental infections from other causes can rigidly be excluded.

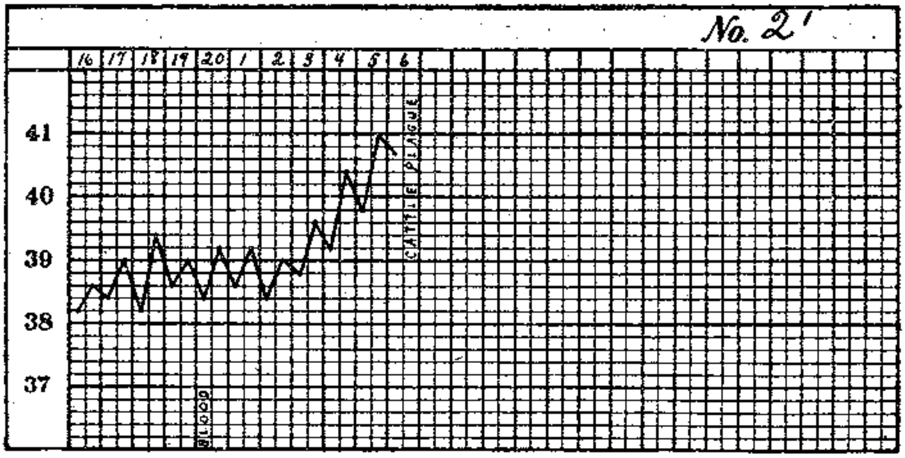
Peritoneal fluid, prepared as above described, appears to be fully as virulent as the blood of the animal. Two serum animals are now being immunized with this fluid, the value of their serum will be compared with that of animals immunized with virulent blood, and the results reported in the near future.

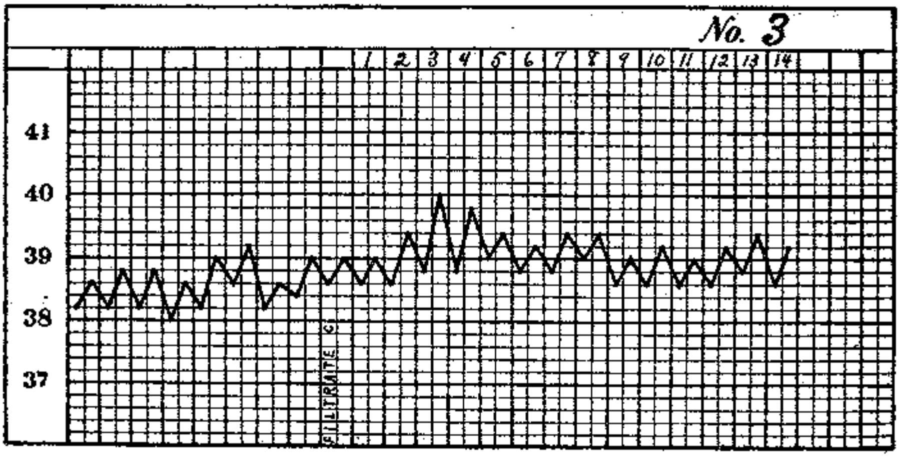
ILLUSTRATIONS.

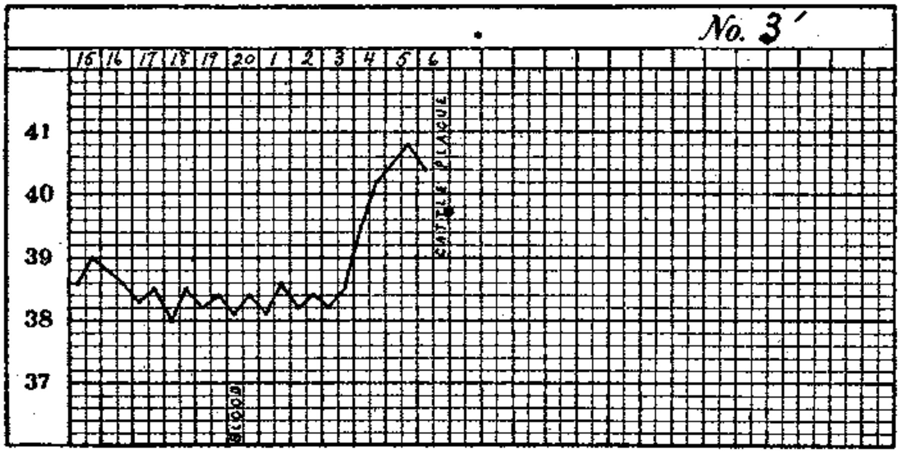
Charts 1 to 16 and 2′ to 11′. 171

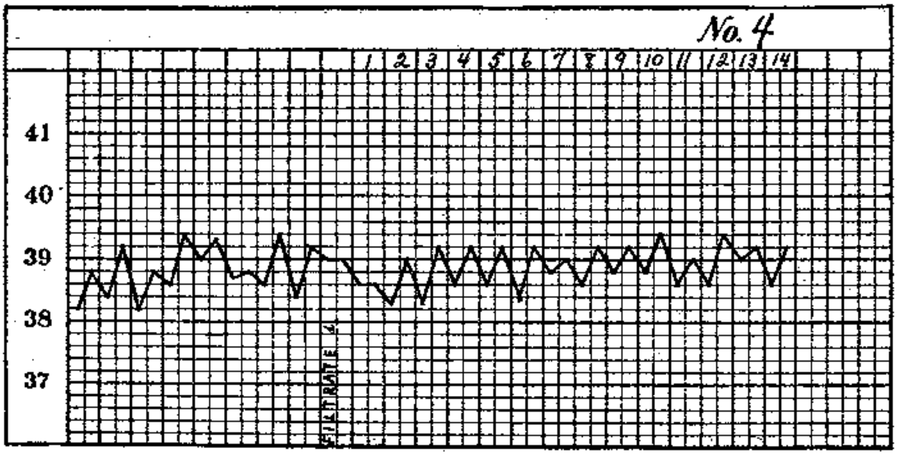

No. 1
1
2
3
4
5
6
7
8
9
10
11
12
13
41
40
39
38
37
BILE
CATTLE PLAGUE

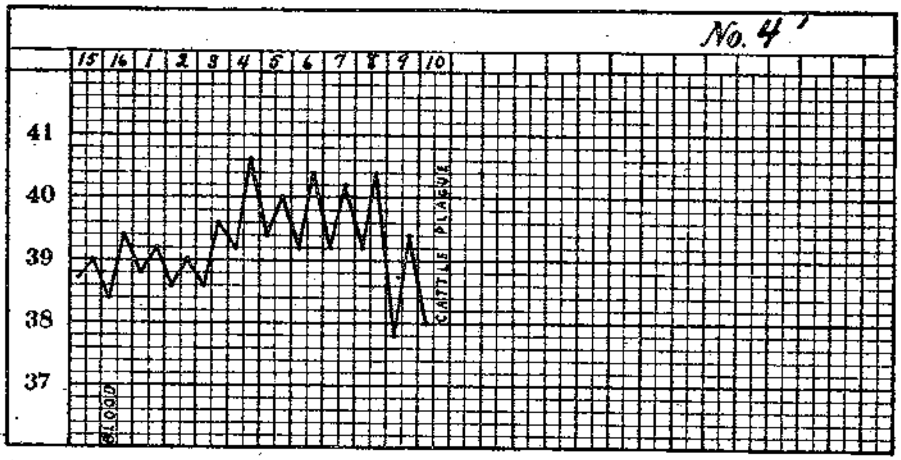

No. 2
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
41
40
39
38
37
FILTRATE 2

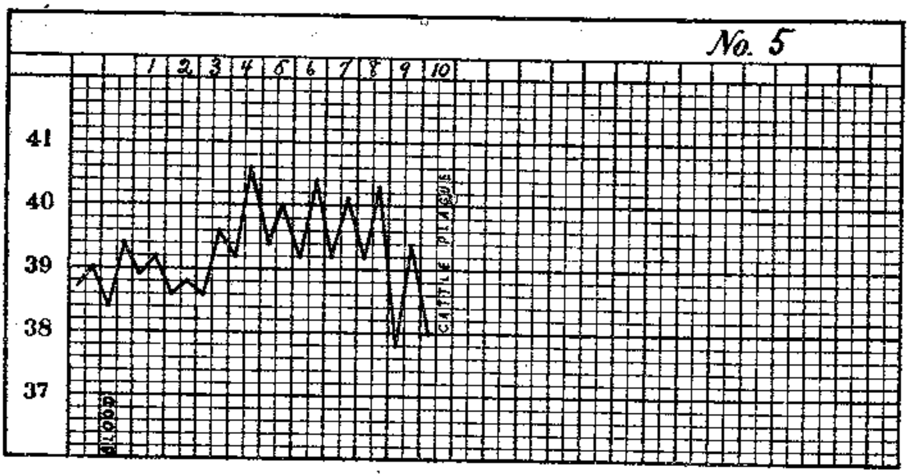

No. 2'
16
17
18
19
20
1
2
3
4
5
6
41
40
39
38
37
BLOOD
CATTLE PLAGUE

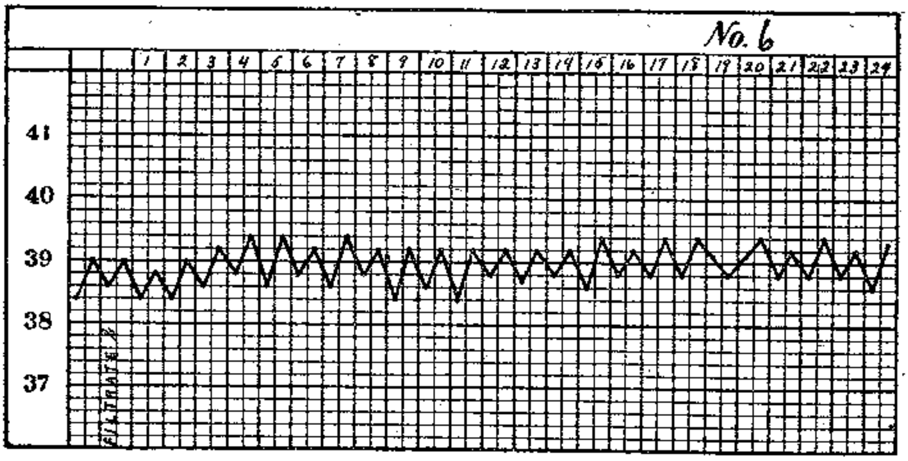

No. 3
1
2
3
4
5
6
7
8
9
10
11
12
13
14
41
40
39
38
37
FILTRATE C

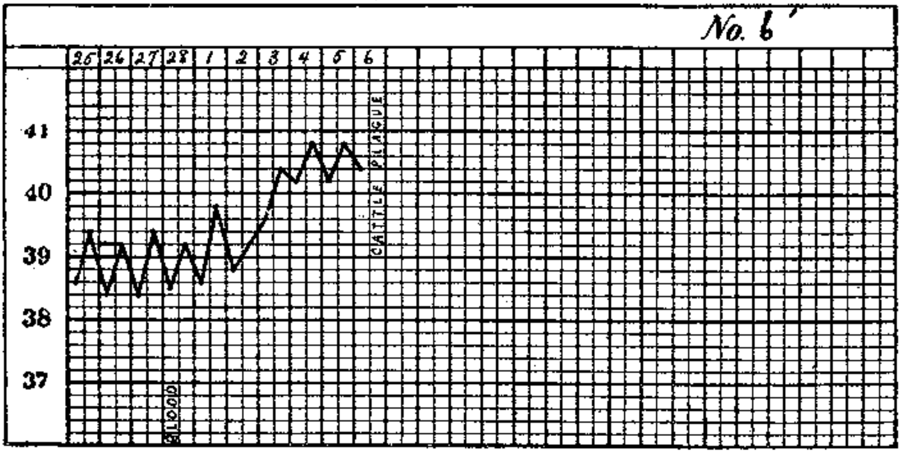

No. 3′
15
16
17
18
19
20
1
2
3
4
5
6
41
40
39
38
37
BLOOD
CATTLE PLAGUE

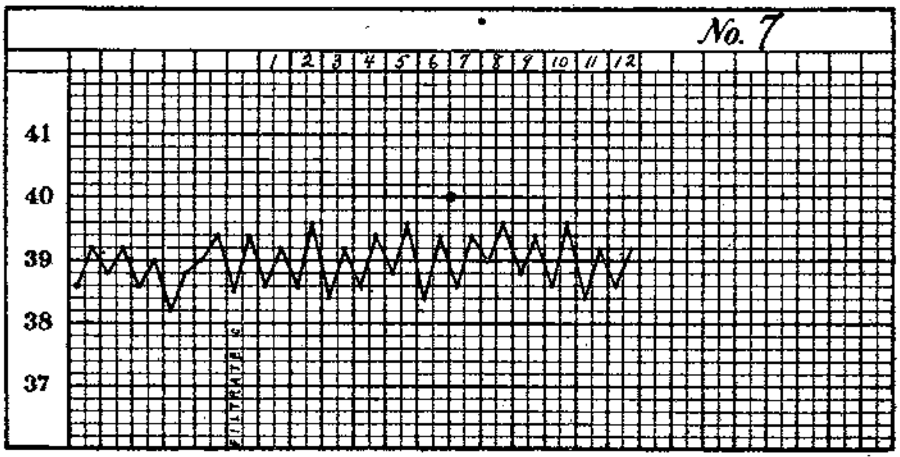

No. 4
1
2
3
4
5
6
7
8
9
10
11
12
13
14
41
40
39
38
37
FILTRATE 4

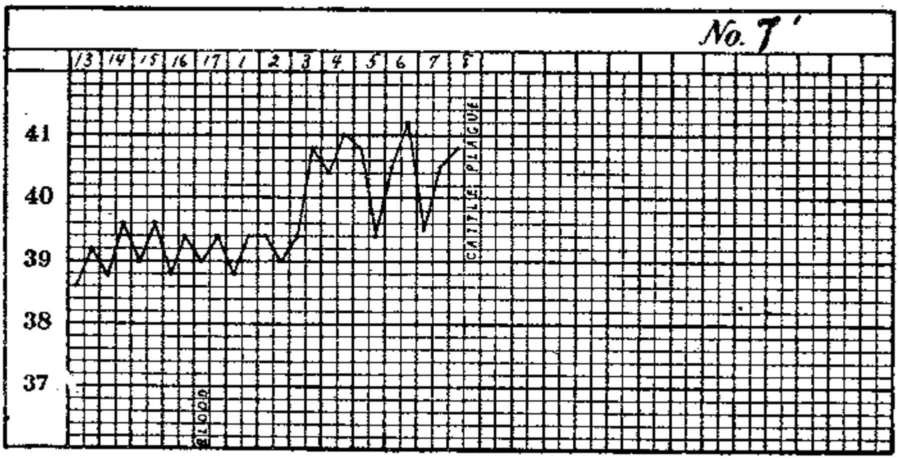

No. 4
15
16
1
2
3
4
5
6
7
8
9
10
41
40
39
38
37
BLOOD
CATTLE PLAGUE

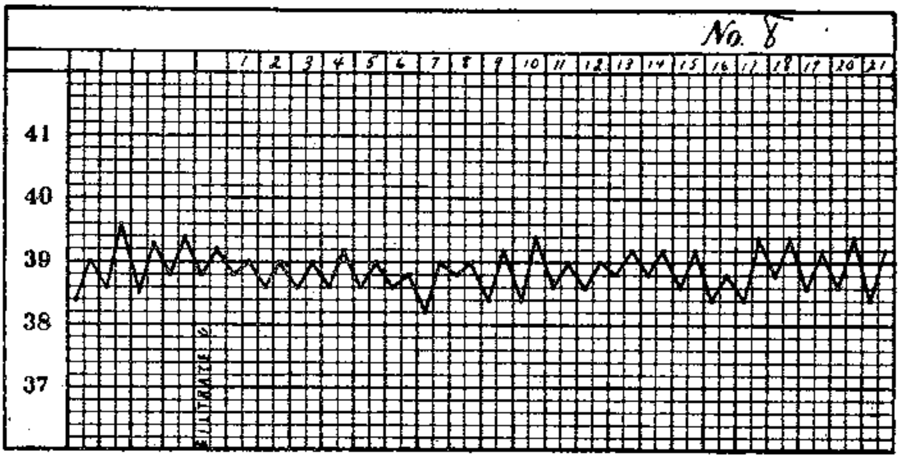

No. 5
1
2
3
4
5
6
7
8
9
10
41
40
39
38
37
BLOOD
CATTLE PLAGUE

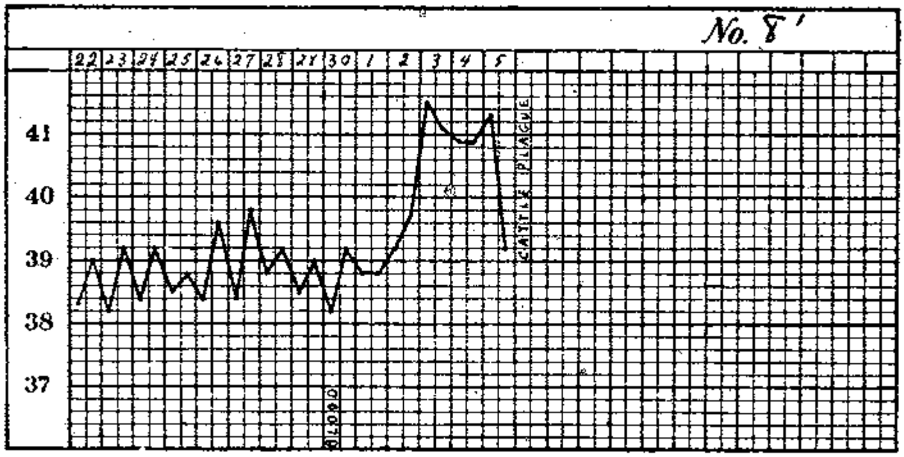

No. 6
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
41
40
39
38
37
FILTRATE B

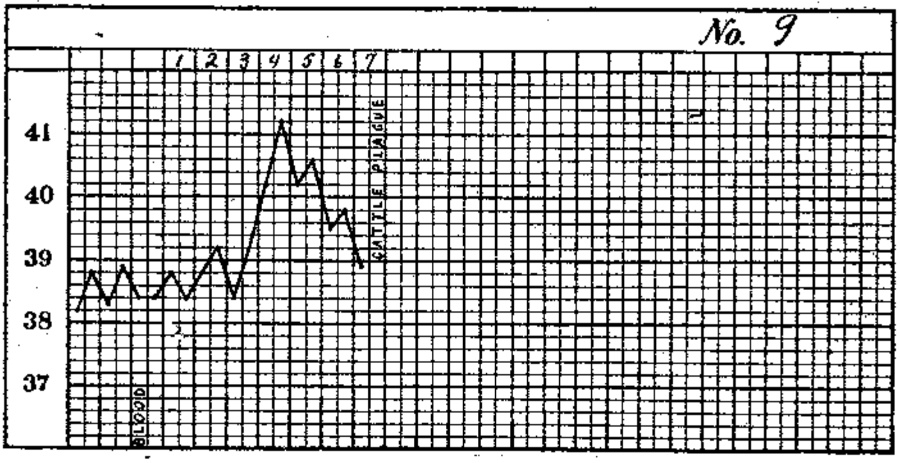

No. 6'
25
26
27
28
1
2
3
4
5
6
41
40
39
38
37
BLOOD
CATTLE PLAGUE

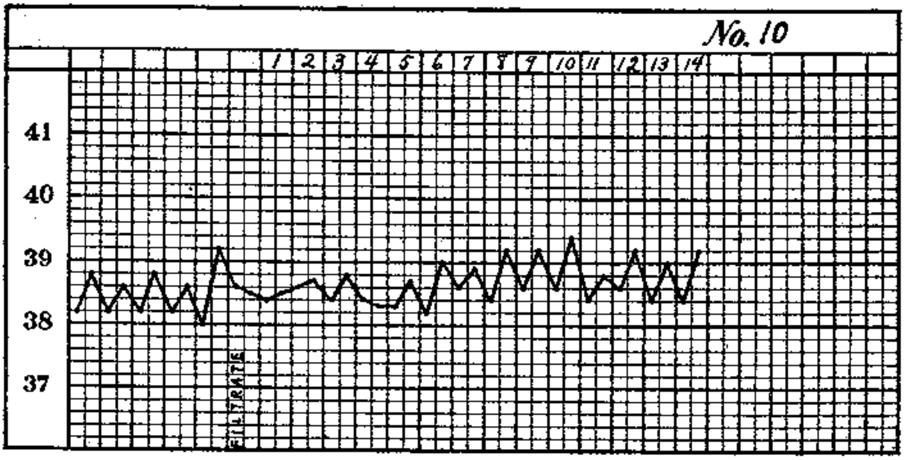

No. 7
1
2
3
4
5
6
7
8
9
10
11
12
41
40
39
38
37
FILTRATE C

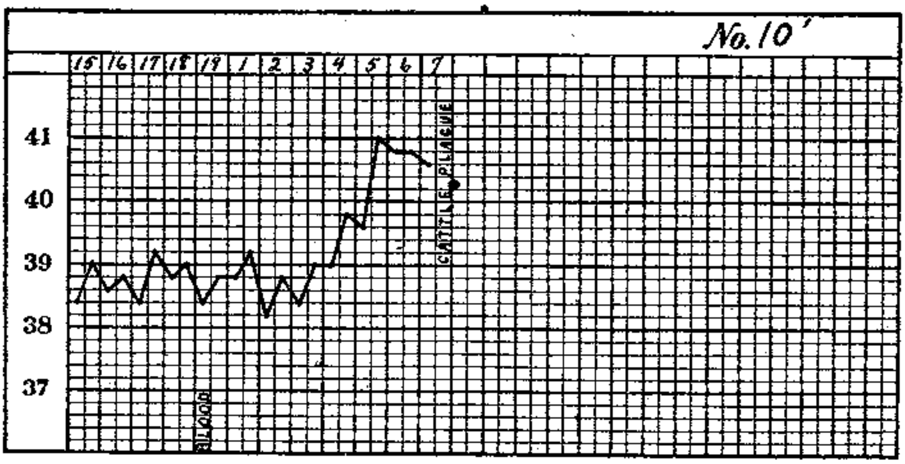

No. 7'
13
14
15
16
17
1
2
3
4
5
6
7
8
41
40
39
38
37
BLOOD
CATTLE PLAGUE

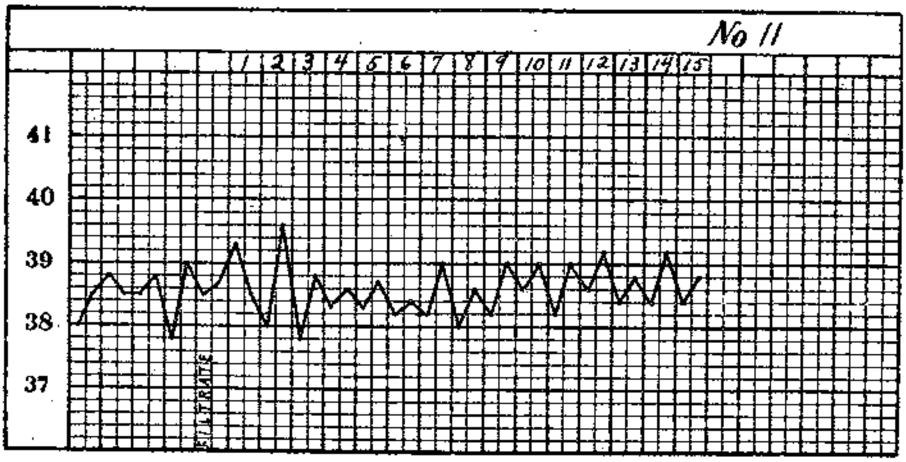

No. 8
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
41
40
39
38
37
FILTRATE D

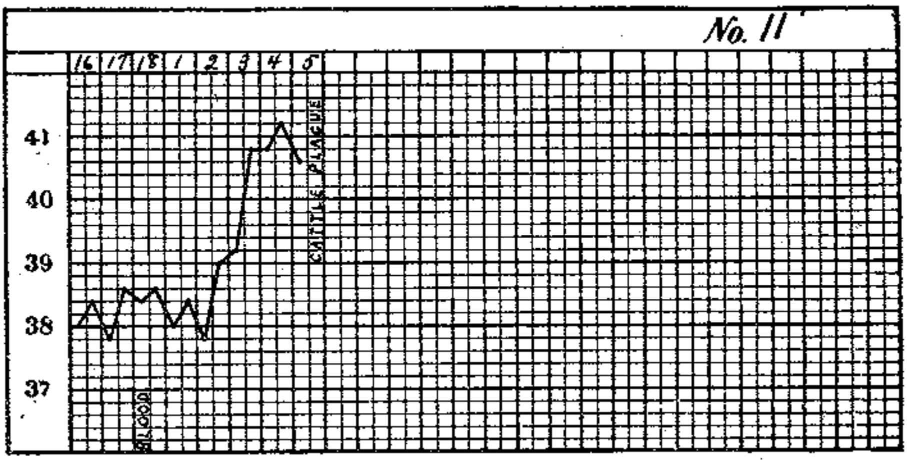

No. 8'
22
23
24
25
26
27
28
29
30
1
2
3
4
5
41
40
39
38
37
BLOOD
CATTLE PLAGUE

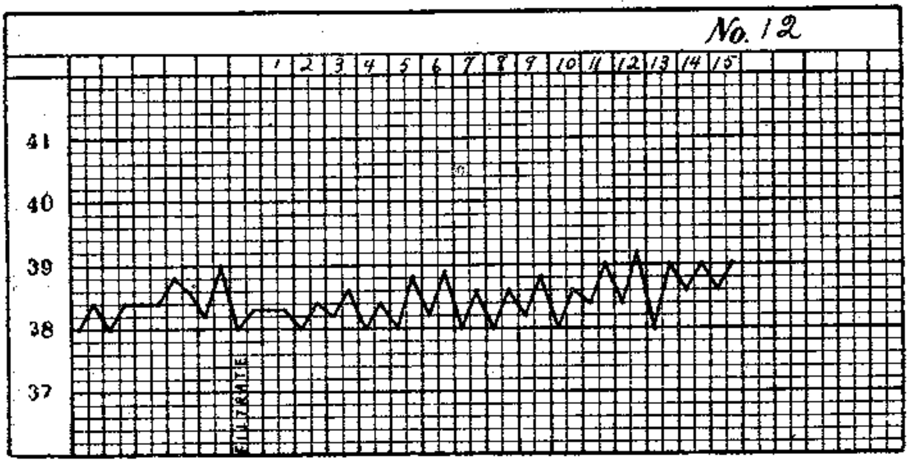

No. 9
1
2
3
4
5
6
7
41
40
39
38
37
BLOOD
CATTLE PLAGUE

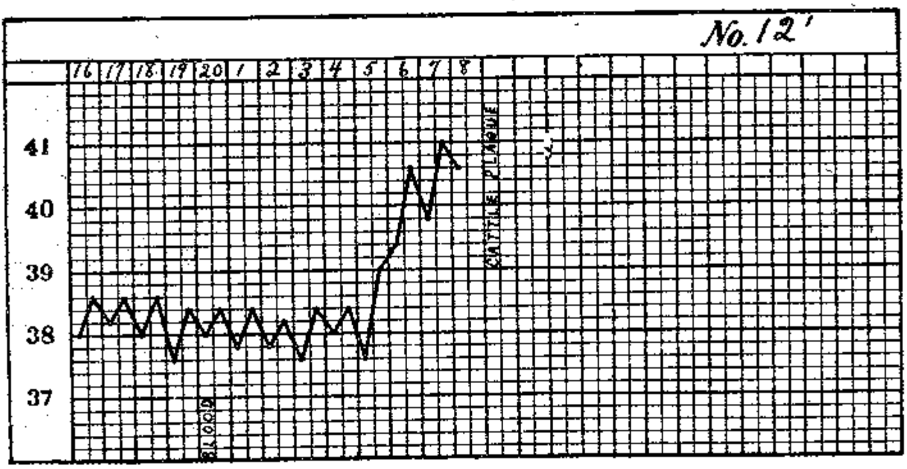

No. 10
1 2 3 4 5 6 7 8 9 10 11 12 13 14
41
40
39
38
37
FILTRATE

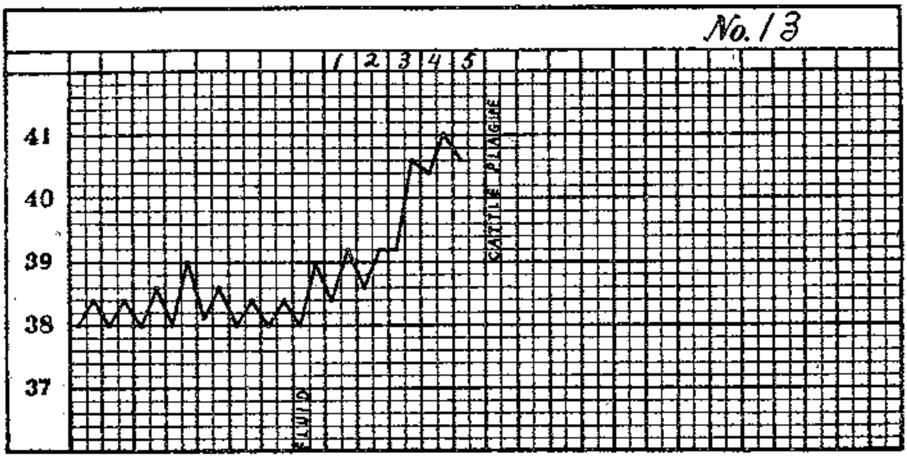

No. 10′
15 16 17 18 19 1 2 3 4 5 6 7
41
40
39
38
37
BLOOD
CATTLE PLAGUE

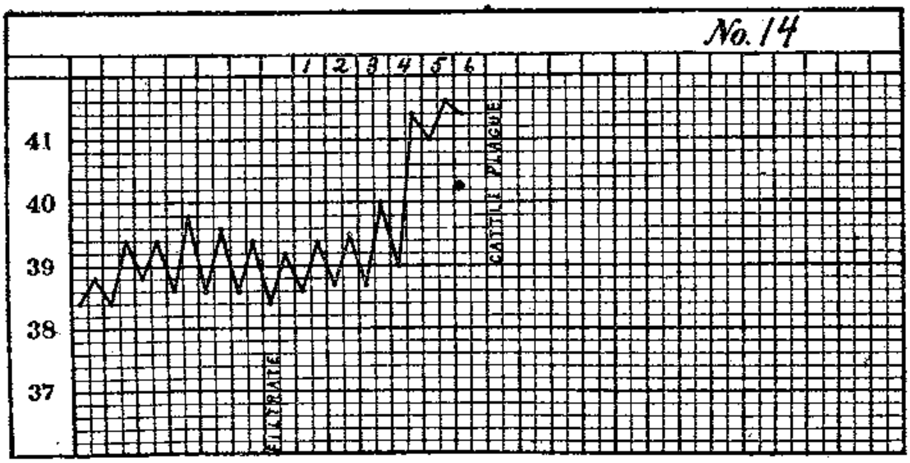

No 11
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
41
40
39
38
37
FILTRATE

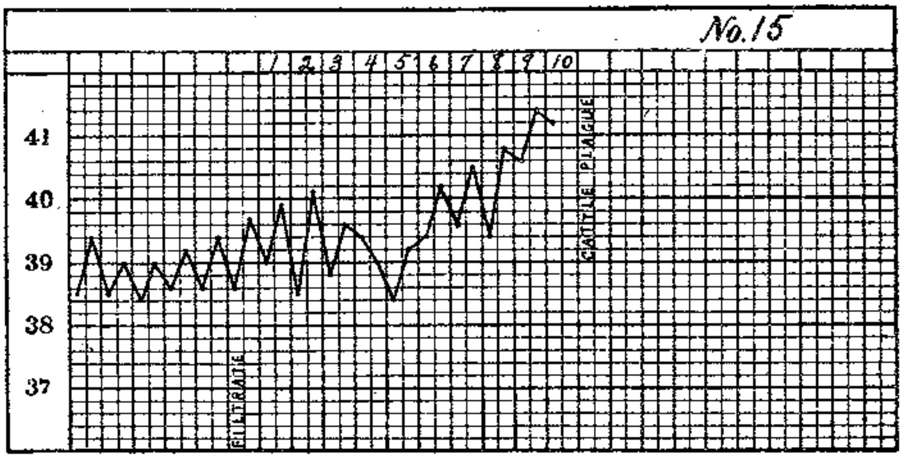

No. 11'
16
17
18
1
2
3
4
5
41
40
39
38
37
BLOOD
CATTLE PLAGUE

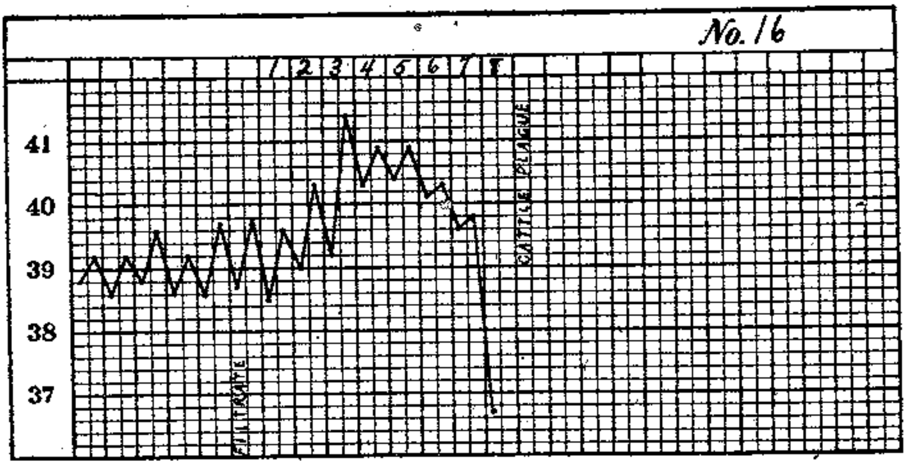

No. 12
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
41
40
39
38
37
FILTRATE


No. 12'
16
17
18
19
20
1
2
3
4
5
6
7
8
41
40
39
38
37
BLOOD
CATTLE PLAGUE


No. 13
1
2
3
4
5
41
40
39
38
37
FLUID
CATTLE PLAGUE


No. 14
1
2
3
4
5
6
41
40
39
38
37
FILTRATE
CATTLE PLAGUE


No. 15
1
2
3
4
5
6
7
8
9
10
41
40
39
38
37
FILTRATE
CATTLE PLAGUE


No. 16
1
2
3
4
5
6
7
†
41
40
39
38
37
FILTRATE
CATTLE PLAGUE

EDITORIAL.

The unification of the medical interests of the Philippine Islands and the harmonizing of the respective attitudes of the American and Filipino physicians has been steadily going on for a number of years.

The Philippine Islands Medical Association had its first annual meeting in 1903, with rather a small attendance and a limited number of papers for discussion. No delegates from other countries were present. Each succeeding year has brought out a larger attendance and a greater interest. In 1906 we had two representatives—one from Hongkong and one from Japan. In 1907 three delegates attended—one from Hongkong, one from Japan and one from China: In 1908 there were eleven, representing respectively: The Imperial Japanese Government, the Imperial Chinese Government, the Imperial Siamese Government, Ceylon, Saigon, the Federated Maylay States, Singapore, and Hongkong.

At the fifth annual meeting, a representation comprising many of the countries included in the so-called territory of the Far East was present and the time was thought to be ripe for the formation of the Far Eastern Association of Tropical Medicine, to meet once in two years upon the invitation of one of the Governments of the component countries. This movement we hope will cement together the medical and scientific fraternities of the territories in question, will stimulate investigation and in addition will bring the center for the publication of research work in Far Eastern tropical medicine where it belongs, namely to some Government or society in the Orient.

Oriental medicine is undergoing a rapid expansion and is attracting increased attention throughout the world. Oriental governments are dealing with peoples whose prejudices and traditions work against the general scientific application of the art of medicine. This attitude must gradually be changed, but the results to be expected are sufficiently great to warrant the outlay of the best energies of the most prominent scientific workers of the world. It means nothing less than an improved hygienic understanding among Oriental peoples and the gradual removal of the causes which underlie certain phases of their stagnation. It will be accompanied by a general movement towards an enlarged sphere of activity among the people. The papers read at the Fifth Annual Meeting of the Philippine Islands Medical Association will be printed in this JOURNAL during 1908 and the discussions on the various topics will be given in its editorial columns. By this system readers can not only become familiar with the experimental data, but also with the opinions of physicians in this portion of the globe.

PAUL C. FREER.

DISCUSSION ON THE PAPERS BY DR. VICTOR G. HEISER AND DR. HARRY T. MARSHALL.

Colonel L. M. Maus, deputy surgeon-general, United States Army: It affords me much pleasure to have occasion to express myself in regard to the practical conditions involved in the spread of cholera, as I had abundant opportunity during the early part of the epidemic in 1902 to study some of the channels of the dissemination of the disease. The history given by Dr. Heiser in regard to its origin agrees exactly with my own experience; it seems to be as nearly a correct one as we could find it to be at that time. A ship's cargo of cabbage came to Manila at the time mentioned and as the health office of Hongkong had notified us of the occurrence of the disease in that city, the Marine-Hospital Service was enabled to reject the shipment, which was dumped into the bay, but notwithstanding, several heads reached the beach at the Farola district and cholera developed. I remember the first case distinctly. It was sent to the San Juan de Dios Hospital; several others soon followed. A number of the members of the medical profession capable of making an accurate diagnosis were present. Cultures were taken from the bowels on the same night and the vibrio was observed under the microscope, but the final diagnosis was not completed until twenty-four hours later. The fact that the cases showed the cholera vibrio to be present and the further fact that there had been no cholera in the Islands up to that time, proved that the disease was imported. As you all know, the provinces were thoroughly supplied with medical officers when the epidemic of 1902 appeared, so that at that time, if there had been any disease in the Archipelago similar to cholera it would have been reported at once, and for that reason I am willing to say that cholera was not present in the Islands at the time of the outbreak in 1902. I believe therefore that the disease in this epidemic was imported *de novo*. It was brought from Hongkong, and spread from the Farola district.

The vibrio of cholera secretes a very virulent toxin. In the course of successive generations of the organism, the infections become milder. It should be noted that the vibrio which was introduced at the beginning of the epidemic in March, 1902, was of the most virulent character. I believe the infection spread largely from fæcal matter through the intervention of flies. Typhoid was stated to be a water-borne disease. In 1898, 300,000 soldiers went to the Spanish-American War, and the medical officers had some knowledge of the results which appeared during the campaign. A large percentage of the troops was infected with typhoid, and we found the soldiers to acquire the disease not only by means of the drinking water, but also by the spread of the organism through the intervention of flies, and I believe cholera to be disseminated in the same way, by flies contaminating the food. It is well known that the natives of the Philippines largely eat with their fingers from a common dish and this custom conduces to the spread of the disease. Owing to their defective habits of personal hygiene, many of the natives have their fingers infected. It is easy to see that their fingers may carry millions of microbes. Flies which carry the fæcal deposits on their feet can take the infection into the markets and thus spread the disease. Anyone who goes into a large military camp in the field will notice that the closets at meal times are frequently free from flies. At meal hours the insects transfer themselves to the tables. This is a fact, and therefore it seems to me that flies in the Philippines also leave the fæcal deposits at times in order to reach the more refined food of the natives. In spite of the general use of distilled water, the wells being closed after the beginning of the outbreak of 1902, little difference in the number of cases was observed. The disease ran its course. I believe the cases now present in the Islands to be sporadic, and that cholera is now endemic.

Dr. Clements, Bureau of Health: It seems to me that the burden of proof in regard to the presence or absence of cholera in the Philippines is upon the man who believes that cholera has disappeared. The great world center of cholera is in the Ganges Valley. I have never been there, but it would seem that the conditions in that region are climatically the same as in this Archipelago. The people are of a comparatively low order of civilization, with very primitive ideas of domestic hygiene living on low, filled lands, no winter breaks the seasons and a very large population is crowded into the area. We are practically certain that cholera never disappears from the Ganges Valley. Therefore I believe that those who maintain that the disease has disappeared from the Philippines must prove this to be the case. Cholera in my belief has been here all the time since it was first introduced. The native in some respects has a fairly good clinical eye. The lowest class of natives know smallpox and measles just as well as we do and these infections have native names. On the other hand, cholera and some other diseases which have been brought into the Archipelago in the early times of Christian occupation are known to the natives only by the Spanish designation, which to my mind is evidence that cholera was introduced since Spanish settlement and was unknown previously. I desire proof that it has ever disappeared after having been once introduced into the Philippines. I have done probably as much field work in connection with cholera as any other man in the Islands. I have been all over the Archipelago and mild cases of a disease which is exactly like cholera, except in the percentage of mortality, appear every year. I have been told this in every place I visited.

In regard to the epidemic of 1902 I think that some of the remarks which have been made here in reference to the probability of variability in the strains of pathogenic organisms are correct. It is very possible that a new strain of cholera organisms was introduced in 1902. The outbreak in 1905, which began in the latter part of August at Jala-Jala (see Map 1 of the paper: "Some consideration with regard to the cause of the frequent reappearance of cholera in the Philippine Islands) was preceded by three nonfatal cases of cholera at Pililla, about 15 miles away. In a barrio of Taguig there had been an epidemic of diarrhœa in June in which the patients showed all the symptoms of cholera, but in which there was no mortality, and time after time I have been told of similar outbreaks. It is a fact that simultaneously with the ripening of the rice crop many cases occur which show symptoms of diarrhœa and sometimes even of cholera; but I do not think we can attribute the disease to the breeding of cholera organisms in this medium, as the water is turned off of the paddies some time before the rice is cut, and the latter is cut dry.

The lower classes of natives in the provinces are very improvident. they live on short rations for about a month just before the new rice is harvested, and after that time, gorge themselves and have a *fiesta* in honor of the recent crop, and diarrhœas are frequent at such times. It may be that new rice is not as digestible as old.

Dr. Heiser, Bureau of Health: I was very much interested in what Colonel Maus said about cholera, in that it brought out many of the difficulties with which we had to contend.

It is generally believed that the outbreak of cholera in 1902 was more severe than that of 1905, while the fact is, which is not generally known, that during the first three weeks of the outbreak in 1905 we actually had more cholera than during the same period in the outbreak of 1902.

One other point is that it has been learned from practical experience that if any community has suffered any cholera within eighteen months, an outbreak there is never so severe as where it has been absent for a longer period. To illustrate this point, we have an outbreak at present in Zambales Province, which has not suffered from cholera for nearly three years, so that the disease may be considered to be epidemic in that province, and the mortality rate is high, while in other provinces which have had cholera during the last eighteen months, the disease is not so severe and the mortality is lower.

It is well known that green rice produces a great number of cases of gastro-intestinal disease, resembling cholera, throughout the Islands. In the rice-harvesting season there are many deaths of this kind. We can not say whether these are due to cholera or not, but it is certainly true that green rice produces cholera-like symptoms. I think the fact brought out by Dr. Clements that the rice field is dry at the time of harvest has certainly made it appear impossible that the cholera organism is propagated in the rice paddies. There is a preparation made from the new rice called *calamay*. It is very attractive to flies, and if there happens to be any cholera in the neighborhood, it is conceivable that this food might easily become infected and become a means of propagating the disease.

Dr. Marshall, Biological Laboratory, Bureau of Science: In concluding I wish to call attention to one statement made by Dr. Heiser to the effect that the cholera vibrio does not seem to abate in virulence while passing through animals and he concluded that some external cause produced the fall of epidemics. My experiments prove that the virulence may diminish most markedly even under favorable conditions of passage through animals.

Ruffer has also shown that the virulence of bacteria, identical with cholera culturally and in other ways, is subject to great variation. In this connection I also wish to call attention to Dr. Clements's statement and emphasize the extreme rapidity with which variation from nonvirulent to virulent bacteria may take place. In experiments with organisms of dysentery a nonagglutinating variant can be produced within the space of a few hours.

Dr. Heiser's hypothesis of gradual immunization through repeated infections with mild or nonvirulent strains of cholera is interesting, but it can not be said at present whether this is a feasible means of immunization or not, possibly, a local immunity of the intestine may result, but the observations do not show that a general immunity may follow. The results of the ingestion of bacteria or of toxins are not conclusive.

In referring to some remarks made by Dr. Musgrave I wish to state that it is usual for the cholera vibrio to be killed in the presence of ordinary saprophytic bacteria, and it is usually stated that it dies rapidly in sewers. I have not encountered in the literature accounts of any experiments in which a symbiotic condition favorable for cholera has been developed.

DISCUSSION OF THE PAPER BY DR. EDWARDS.

Captain Ryley, R. A. M. C., Hongkong: When an epidemic occurs in tropical countries it is usual for the people to drink distilled water or soda water. I inoculated soda water artificially with cholera organisms, and I found that the usual pressure of about 150 pounds to the square inch, which it is believed should destroy pathogenic bacteria, had no effect on the cholera vibrio. The soda water was of the variety which is made by impregnating the water with carbonic-acid gas. I was able to recover the cholera organism from the water which I worked with.

Dr. Carpenter, United States Navy: At the Cavite Naval Station for the past year we have had experience with artesian well water. We found that the water which came from a depth of 508 feet was infected when it first came through the pump and we began to employ boiled water. This was not sufficient to supply several thousand people and we then built a distilling plant which has no equal in these Islands. Each tank before filling is sterilized, the water drawn off into galvanized cans with tightly fitting covers the next day, the latter being washed with live steam each time they are filled. During the last three years the records of this station show the percentage of infection of men with amœbic dysentery to be from 0.8 per cent to 1.5 per cent. The first quarter after distilled water was used the percentage was 5.8, and 4.8 per cent for the next quarter.

I also desire to point out that the percentage of infection with amœbæ at Fort McKinley among the troops using distilled water was formerly 70 per cent. At Canacao, the water became contaminated by amœbæ and attempts were made to remove the cause, but these were finally abandoned because the mains would immediately be reinfected. It is my belief that there are many other ways in which amœbic infection can be acquired in addition to that by means of drinking water. There are many people on this station who use ordinary water, and apparently these are no more infected than those who only use distilled water.

DISCUSSION ON THE PAPER BY DR. T. KITAJIMA.

Mr. Charles S. Banks, Bureau of Science: I would like to say for the information of those present that we have nine species of this genus in the Philippines, the commonest (*Trimesurus hypnale* D. & B.), a green snake with yellow belly, is called "alimurain-alupon" by the natives.

Dr. Harry T. Marshall, Bureau of Science: I wish to congratulate Professor Kitajima upon his interesting paper. He has been able to establish the fact that the habu venom acts according to the same general laws that govern in the cases of the cobra venom, rattle-snake venom and other animal poisons. The action of his antiserums is promising, and they will be beneficial in proportion to his success in securing their general employment.

REVIEWS.

The Treatment of Disease, a Manual of Practical Medicine. By Reynold Webb Wilcox, M. A., M. D., LL. D. Cloth. Pp., xxviii+911. Price $6. Philadelphia: P. Blakiston's Son & Co., 1907.

Comparative Anatomy of Vertebrates. Adapted from the German of Dr. Robert Wiedersheim by W. N. Parker, Ph. D. Third edition, founded on the sixth German edition. With three hundred and seventy-two figures and a bibliography. Cloth. Pp. xii + 576. Price $3.75 net. New York: The Macmillan Company, 1907.

The scope of this work is limited to what is signified in its title, and the subject is treated tersely and clearly from the standpoint of evolution. After the introduction, which gives a synopsis of the early stages of development in general, the different systems are presented briefly with definitions of terms beginning with the integument followed by the skeleton, muscles, etc.; then each system is described as found in *Amphyoxus*, in fishes, in amphibians, in reptiles, in birds and in mammals. The arrangement is simple and convenient for students in its use as a text book. Many excellent, well-selected drawings increase its efficiency. A ready index and a bibliography add to its value as a reference work, although meager in details and too brief to be of great value to investigators. The style is clear and limpid, concise and pointed as a rule, but there are obscure passages that need reconstruction, as for example on page 122, beginning with line 2. "The cranial cavity has become further enlarged at the cost of parts formerly situated extracranially than is the case among Reptiles."

The emphasis placed on evolution necessitates ontogenetic and phylogenetic details and paleontological records that force the question as to the reason for many striking similarities of extinct forms to existing ones, and of these to each other in both the development of the individual and of the race. Is this similarity to be explained by evolution, or is it only a matter of the same laws controlling development, so that in one this development reaches beyond the point of its cessation in others, thus leaving traces of the early stages as rudiments, vestiges, degenerations, etc.? The latter explanation is as plausible and workable as the former, and does not require "blood relationships of animals in general" as a factor.

The treatment of the segmentation of the ovum is crude and simple. It might have been improved by a few plates illustrating the various steps of cell cleavage by mitosis.

The arguments for the vertebral theory of the skull are given and tacitly agreed upon as plausible, although they have been repeatedly shown to be unreal. The presence of the "os centrale" in the human embryo and other facts of a similar nature are not emphasized in relating man to lower forms, although the *adapter* is continually relating the different animals forms with one another. A serious omission is found on page 249 in the enumeration of the senses, where the muscle sense is not even mentioned, and pleasure and pain sensations are denied a place. There is no suggestion of visceral sensations, the "yearning of the bowels," that is so real in man.

But one learns a great deal by further perusal when on page 393 the capillaries are said to be "surrounded by contractile structures analogous to the smooth muscle-fibers of the larger vessels and consisting of branched muscle-cells which are under the control of the nervous system."

However, the climax is reached on page 492 where the adrenal bodies are treated of in connection with the urinogenital organs because of the "difficulty of knowing in what other connection to describe them." This brings us to a serious consideration of the classification of the so-called ductless glands, or the glands furnishing *internal secretions* to the blood. Anatomists might safely follow the physiologists and arrange such glands under the head of ductless glands. The following scheme is presented with the hope that it will clear up some obscurity in regard to the internal secretions, and serve as a ready means of facilitating the learning of the *thirteen ductless glands* of the *human* body.

ACCESSORY BLOOD GLANDS.

I. PRODUCE INTERNAL SECRETION.

a. Ductless glands: Thyroids, parathyroids, adrenal bodies, pituitary body.

b. Glands with ducts: Liver, kidney, pancreas, testicles, ovaries.

c. Other glands: Stomach, intestines.

II. FUNCTION UNDECIDED.

a. Ductless glands: Spleen, thymus, pineal body, carotids, coccygeal.

b. Glands with ducts.

c. Other glands: Lymph, hæmolymph, bone marrow?

ROBERT BENNETT BEAN.

○

PREVIOUS PUBLICATIONS OF THE BUREAU OF GOVERNMENT LABORATORIES—Concluded.

(Concluded from second page of cover.)

No. 32, 1905.—Biological Laboratory: I. Intestinal Hæmorrhage as a Fatal Complication in Amœbic Dysentery and Its Association with Liver Abscess. By Richard P. Strong, M. D. II. The Action of Various Chemical Substances upon Cultures of Amœbæ. By J. B. Thomas, M. D., Baguio, Benguet. *Biological and Serum Laboratories:* III. The Pathology of Intestinal Amœbiasis. By Paul G. Woolley, M. D., and W. E. Musgrave, M. D.

No. 33, 1905, Biological Laboratory.—Further Observations on Fibrin Thrombosis in the Glomerular and in Other Renal Vessels in Bubonic Plague. By Maximilian Herzog, M. D.

No. 34, 1905.—I. Birds from Mindoro and Small Adjacent Islands. II. Notes on Three Rare Luzon Birds. By Richard C. McGregor.

No. 35, 1905.—I. New or Noteworthy Philippine Plants, IV. II. Notes on Cuming's Philippine Plants in the Herbarium of the Bureau of Government Laboratories. III. Hackel, "Notes on Philippine Grasses." IV. Ridley, "Scitimineæ Philippinenses." V. Clarke, "Philippine Acanthaceæ." By Elmer D. Merrill, Botanist.

No. 36, 1905.—A Hand-List of the Birds of the Philippine Islands. By Richard C. McGregor and Dean C. Worcester.

The previous publications of the Bureau were given out as bulletins in serial number pertaining to the entire Bureau. These publications, if they are desired, can be obtained by applying to the librarian of the Bureau of Science, Manila, P. I., or to the Director of the Bureau of Science, Manila, P. I. Correspondents will confer a favor by returning to the Bureau any previous publications which they may have in duplicate, as a number of bulletins are now out of print.

LIST OF PREVIOUS PUBLICATIONS OF THE MINING BUREAU (NOW DIVISION OF MINES OF THE BUREAU OF SCIENCE).

1890.—Descripción física, geológica y minera en bosquejo de la Isla de Panay por D. Enrique Abella y Casariego, Inspector General de Minas del Archipiélago.

1890.—Memoria descriptiva de los manantiales minero-medicinales de la Isla de Luzon, estudiados por la comisión compuesta de los Señores D. José Centano, Ingeniero de Minas y Vocal Presidente, D. Anacleto del Rosario y Sales, Vocal Farmacéutico, y D. José de Vera y Gómez, Vocal Médico.

1893.—Estudio Descriptivo de algunas manantiales minerales de Filipinas ejecutado por la comisión formada por D. Enrique Abella y Casariego, Inspector General de Minas, D. José de Vera y Gómez, Médico, y D. Anacleto del Rosario y Sales, Farmacéutico; precidido de un prólogo escrito por el Excmo. Sr. D. Angel de Avilés, Director General de Administración Civil.

1893.—Terremotos experimentados en la Isla de Luzón durante los meses de Marzo y Abril de 1892, especialmente desastrosos en Pangasinán, Unión y Benguet. Estudio ejecutado por D. Enrique Abella y Casariego, Inspector General de Minas del Archipiélago.

1901.—The Coal Measures of the Philippines. Charles H. Burritt.

1902.—Abstract of the Mining Laws (in force in the Philippines, 1902). Charles H. Burritt.

1902, Bulletin No. 1.—Platinum and Associated Rare Metals in Placer Formations. H. D. McCaskey, B. S.

1903.—Report of the Chief of the Mining Bureau of the Philippine Islands. Charles H. Burritt.

1903, Bulletin No. 2.—Complete List of Spanish Mining Claims Recorded in the Mining Bureau. Charles H. Burritt.

1903, Bulletin No. 3.—Report on a Geological Reconnoissance of the Iron Region of Angat, Bulacan. H. D. McCaskey, B. S.

1904.—Fifth Annual Report of the Mining Bureau. H. D. McCaskey.

1905.—Sixth Annual Report of the Chief of the Mining Bureau. H. D. McCaskey.

1905, Bulletin No. 4.—A Preliminary Reconnoissance of the Mancayan-Suyoc Mineral Region, Lepanto, P. I. A. J. Eveland, Geologist.

1905, Bulletin No. 5.—The Coal Deposits of Batan Island. Warren D. Smith, B. S., M. A., Geologist.

LIST OF PREVIOUS PUBLICATIONS OF THE ETHNOLOGICAL SURVEY (NOW DIVISION OF ETHNOLOGY, BUREAU OF SCIENCE).

(For sale at Bureau of Printing.)

Vol. I.—The Bontoc Igorot, by Albert Ernest Jenks. Paper, ₱4.50; half Morocco, ₱7.

Vol. II, Part 1.—Negritos of Zambales, by William Allen Reed. Paper, ₱1.25; half Morocco ₱3.75.

Vol. II, Part 2 and Part 3.—The Nabaloi Dialect, by Otto Scheerer. The Bataks of Palawan, by Edward Y. Miller. (Bound also in one volume with Part 1, Negritos of Zambales.) Paper, ₱1.25; half Morocco, ₱3.75. Combined half Morocco, ₱5.

Vol. III.—Relaciones Agustinianas de las razas del Norte de Luzon, by Perez. Not listed by Bureau of Printing.

Vol. IV, Part 1.—Studies in Moro History, Law, and Religion, by Najeeb M. Saleeby. Paper, ₱0.75; half Morocco, ₱3.25.

---

[1] The first four bulletins in the ornithological series were published by The Ethnological Survey under the title "Bulletins of the Philippine Museum." The other ornithological publications of the Government appeared as publications of the Bureau of Government Laboratories.

CONTENTS

Page.

MUSGRAVE, W. E. The Influence of Symbiosis Upon the Pathogenicity of Microörganisms ... 77

HEISER, VICTOR G. Some Considerations with Regard to the Cause of the Frequent Reappearance of Cholera in the Philippine Islands (with Statistics Beginning with the Outbreak in 1902 to January 1, 1908) ... 89

MARSHALL, HARRY T. Studies of Cholera ... 107

EDWARDS, RALPH T. A Biological Study of the Water Supply of the Philippine Islands, with a Description of a New Pathogenic Organism ... 121

YERSIN, A., and VASSAL, J. J. Typhus Fever in Indo-China ... 131

KITAJIMA, T. On "Habu" Venom and its Serum Therapy ... 151

RUEDIGER, E. H. Filtration Experiments with the Virus of Cattle Plague ... 165

EDITORIAL ... 183

REVIEWS ... 189

The "Philippine Journal of Science" is issued as follows:

Section A. General Science, $2, United States currency, per year.
Section B. Medical Sciences, $3, United States currency, per year.
Section C. Botany, $2, United States currency, per year.

The entire "Journal," $5, United States currency, per year.
Single numbers, 50 cents, United States currency.
Authors receive 100 copies of their paper free.

The numbers in each section will appear as rapidly as material is available. Each section will be separately paged and indexed. Subscriptions may be sent to the DIRECTOR OF PRINTING, Manila, P. I.

FOREIGN AGENTS.

THE MACMILLAN COMPANY, 64-66 Fifth Avenue, New York.
Messrs. WM. WESLEY & SON, 28 Essex Street, Strand, London, W. C.
MARTINUS NIJHOFF, Nobelstraat 18, 's Gravenhage, Holland.
Messrs. MAYER & MÜLLER, Prinz Louis Ferdinandstrasse 2, Berlin, N. W.
Messrs. KELLY & WALSH, LIMITED, 32 Raffles Place, Singapore, S. S.
Messrs. A. M. & J. FERGUSON, 19 Baillie Street, Colombo, Ceylon.

(Entered at the Post-Office at Manila, P. I., as second-class matter.)

A limited number of complete copies of Volume I, Supplement to Volume I, and Volume II, all sections, are still available for sale. Price of the complete volumes unbound, each, $5, United States currency; the Botanical Supplements to Volume I unbound, $2.50, United States currency. Volume I was not divided into sections.